FAR EASTERN ASSOCIATION OF TROPICAL MEDICINE

TRANSACTIONS OF THE SEVENTH CONGRESS

FAR EASTERN ASSOCIATION OF TROPICAL MEDICINE

TRANSACTIONS

OF THE

SEVENTH CONGRESS

HELD IN

BRITISH INDIA

DECEMBER 1927

EDITED BY

LT-COL J CUNNINGHAM CIE BA MD IMS

Director Pasteur Institute of India

Kasauli British India

General Organizing Secretary for the Seventh Congress

VOLUME III.



Published for

THE SEVENTH CONGRESS

By

THACKERS PRESS & DIRECTORIES LTD

6 Mangoe Lane CALCUTTA

Printed by
THACKER'S PRESS & DIRECTORIES LTD
6 Mangoe Lane Calcutta.

CONTENTS

(Volume III)

SECTION IV—(*Continued*)

	PAGE
CONTENTS	v
LIST OF ILLUSTRATIONS	xi
LIST OF SCIENTIFIC SECTIONS	xiii
CHAIRMEN AND RAPPORTEURS OF SECTIONS IV V AND VI	xiv

Kala azar

1 The Kala azar Transmission Problem and the Factor of Resistance by Lieut Col R Knowles I M S — 1

2 The Life history of *Leishmania donovani* in its Insect and Mammalian Hosts by Major H E Shortt I M S — 12

3 Kala azar Studies in North China by Dr Charles W Young and Dr Marshall Hertig — 19

Discussion—Young Hu Brug Shortt Sarkar (S L) Napier Megaw, Ross Ganguly (L B) Brahmachari (U N) Gharpure Christophers Stephens Young Shortt and Knowles — 23

4 The Experimental Transmission of Oriental Sore (causing Generalized Infection) in Laboratory Animals by Dr B M Das Gupta — 34

5 The Action of the Pentavalent Compounds on the *Leishmania donovani* Parasites by Lieut Col H W Acton I M S and Lieut Col R N Chopra I M S — 36

6 The Clasmatocyte in Experimental Kala azar by Dr J R Cash and Dr C H Hu — 44

7 Studies upon the Peripheral Blood Bone marrow and Spleen of Hamsters experimentally infected with Kala azar by Dr C H Hu and Dr J P Cash — 62

8 The Presence of *Leishmania donovani* in the Skin and Subcutaneous Tissue in Cases of Kala azar by Dr J R Cash and Dr C H Hu — 77

9 Erosion of the Inner Table of the Skull by Hyperplasia of Bone marrow in Kala-azar with Extra medullary Formation of Blood on the Surface of the Dura by Dr C H Hu and Dr J P Cash — 80

10 Peripheral Lesions Produced by *Leishmania donovani* and Allied Organisms by Dr Charles W Young and Dr Marshall Hertig — 87

11 Diagnostic Value of the Antimony Test in Kala-azar by Lieut Col R N Chopra I M S Dr J C Gupta and Dr N K Basu — 89

PAGE

Medical Entomology

12 The Morphology of the Buccal Cavity of the Mosquito by Captain P J Barraud and Major G Covell, I M S — 98
13 Some Anopheles of Sarawak by Dr V A Stookes, M C — 103
14 Regional Distribution of Anophelines and Malaria in Bengal by Mr M O T Iyengar — 116
  Discussion —Sinton Senior White, Malcolm Watson, Dunn and Iyengar (M O T) — 126
15 Parasitic Nematodes of *Anopheles* in Bengal by Mr M O T Iyengar — 128
  Discussion —Christophers Sinton, Senior White, Shortt and Iyengar (M O T) — 133
16 Microsporidian Parasites of Anopheles Larvæ by Mr M O T Iyengar — 136
  Discussion —Dalal, Roy (D N), Swaminath, Senior White and Iyengar (M O T) — 142
17 The Anopheles of the Australian Region their Bionomics and their Distribution by Mr Frank H Taylor — 143
18 Nomenclature des Moustiques de la Cochinchine et du sud Annam par Dr E Borel — 165
19 Sur les Moeurs des Anopheles en Cochinchine par Dr E Borel — 169
20 The Identification and Classification of the Species of the Genus *Phlebotomus*, with some Remarks on their Geographical Distribution in Relation to Disease by Major J A Sinton V C, O B E, I M S — 172
21 The Breeding of Sandflies in Nature and in the Laboratory by Dr R O A Smith, I M D — 182
  Discussion —McCombie Young and Smith — 185
22 Study on the Seasonal Prevalence of House Flies in Chosen (Korea) by Dr Harujiro Kobayashi — 186
  Discussion —Senior White — 195

Helminthology

23 Endo parasites du Tarabagan par Dr Li Yuan Po — 196
24 Documents Statistiques sur les Helminthiases à Pondichery par Major V G F Labernadie — 198
25 Strongyloidosis and its Treatment with Gentian Violet by Dr Kwa Tjaon Sioe — 200
26 The Present Status of *Sauricola* and *Echinopharynx* by Dr Govind Singh Thapar — 205
27 Studies on Prophylaxis of Clonorchiasis by Dr Kanji Nagano — 211
28 The Correlation between the Size of Hookworm Egg Counts and the Degree of Anæmia in Two Groups in Southern India by Dr J F Kendrick — 216
29 Notes on a Quantitative Hookworm Survey of Ceylon by Dr W C Sweet — 239
30. The Relation of Ankylostome Infestation to the Physical Features of an Agricultural Area in India and to the Social and Economic Status of its Population by Dr Vishnu T Korke — 249

PAGE

31 The Correlation between Ankylostome Disease and Hæmoglobin Value as obtained in regard to the Physical Features of an Agricultural Area in India and to the Social and Economic Status of its Population by Dr Vishnu T Korke 259

32 Preliminary Observations on Ankylostomiasis in Pariah Dogs by Dr Govind Singh Thapar 265

Discussion —Edwards Stott Maplestone Heiser Kobayashi Tirumurti Kendrick and Korke 265

33 Parasitic Infections in the Foochow Area Fukien Province China by Dr Ernest Carroll Faust and Claude P Kellogg 268

34 *Filaria malayi n sp* parasitic in Man in the Malay Archipelago by Dr S L Brug 279

35 Filarial Infection and Diseases due to *Filaria bancrofti* by Dr S Sundar Rao 299

36 Prevalence of Filariasis in some Areas in British India by Dr Vishnu T Korke 305

Discussion King Pandit Tirumurti Basu (S C) Brug and Korke 309

SECTION V

Diseases of Nutrition

37 Diseases of Faulty Nutrition by Lieut -Col P McCarrison C I E I M S 311

Discussion —Russell 318

38 Normal Basal Metabolism of Indians by Major S S Sokhey I M S 321

Discussion —Mukherjee (H N) 321

39 Relative Values of the National Diets of Certain Indian Races by Lieut Col R McCarrison C I E I M S 322

40 Effects of Faulty Food Deficient in Vitamins on the Gastro intestinal Tract by Lieut Col R McCarrison C I E I M S 324

Discussion B (C J) Edwards Vedder and McCarrison 326

41 A Note on Iodine Metabolism by Major Clive Newcomb I M S and Dr Ganapati Sankaran 329

Discussion —McCarrison and Newcomb 333

42 A Preliminary Note on the Interrelationship of Some of the Endocrine Glands in Sugar Metabolism by Dr J P Bose 335

43 Effect of Manganese on Growth by Lieut Col R McCarrison C I E I M S 343

44 The Experimental Production and Prevention of Stone in the Bladder in Rats by Lieut Col R McCarrison C I E, I M S 345

Discussion —Heiser Bose (C L) Tirumurti Vedder Menkel Sharma, Ganguli (P) Devidasani and McCarrison 346

Deficiency Diseases

45 Epidemic Dropsy Its Bearing on the Beri beri Problem by Lieut Col J W D Megaw C I E V H S I M S 349

		PAGE
46	Beri-beri Columbarum by Lieut-Col R McCarrison, C I E, I M S ..	361
	Discussion—Vedder, Shaha, Jolly, Bentley, Bose (C L), Gloster, Sprawson, Rosedale, Stott, Kelsall, Dutt (S C), Heiser, McCarrison and Megaw	363
47	Prophylaxis and Cure of Beri-beri by Vitamin-preparations by Dr B C. P. Jansen and Dr. W F Donath	372
	Discussion—Rosedale and Jansen ..	374
48.	An Investigation to Determine a Satisfactory Standard for Beri beri Preventing Rices by Lieut-Col Edward B Vedder and Dr R T Feliciano .	375
49.	*Experimental Studies on Injuries to Pigeons caused by Rice of Different Ages and Various Milling Processes* by Dr A Kessler	409
50	Report of the Committee on Beri-beri of the Philippine Islands ..	423
51	The Causation of Lathyrism in Man by Lieut-Col Hugh W Acton, I M S, and Lieut-Col. R N Chopra, I M S	444
	Discussion—McCarrison and McCombie Young . ..	448
52	Salt Licks by Major Clive Newcomb, I M S . .	450
	Discussion—Edwards and Newcomb	452
53	The *Experimental Production of Lymph adenoid Goitre* by Lieut-Col R McCarrison, C I E, I M S	453
54	Relationship of Iodine in Soil and Drinking water to the Chronic Hypertrophic Type of Endemic Goitre by Lieut-Col R McCarrison, C I E, I M S	455
	Discussion—Tirumurti and McCarrison	456

Immunology and Chemotherapy

55	On Some Factors Influencing the Therapeutic Value of the Solutions of Salvarsans by Dr Sahachiro Hata	458
56	The Relation between Chemical Constitution of Antimonials and their Therapeutic Properties by Rai Bahadur Dr U N Brahmachari ..	468
57.	Chemotherapy of Bubonic Plague by Rev Father J F Caius and Dr B P. B Naidu	481
	Discussion—Gupta (A), Sarkar (S L), Napier, Hata and Brahmachari (U N)	488
58	Further Evidence on Lipoidophile Antigen-Antibody Reaction by Dr Tenji Taniguchi	490
59	Development and Duration of Immunity by Inoculation and Re inoculation by Lieut-Col W F Harvey, C I E, I M S (*Retd*) and Captain K R K Iyengar, I M S	503
60	The Treatment of Amœbic Dysentery with Acridin Dyes by Dr O Urchs .	512
61	Some Clinical Aspects of the Wassermann Test (Experience in Calcutta) by Lieut-Col R B. Lloyd, I M S . ..	517
62	Les Methodes de Vernes en Général et la Syphilimétrie en Particulier par Major V G F Labernadie	524

PAGE

63 La Reaction de Bordet Wassermann sans Ftuve par Major V G F Labernadie 527
Discussion—Stott Taniguchi Hata Iyengar (K R K) Lloyd and Labernadie 5?8

Rabies and Anti rabic Treatment

64 The Action of Ether on the Rabies Virus by Lieut Col J Cunningham I M S, Drs M J Nicholas I M D and B N Lahiri I M D 531
Discussion—Parker Hitchens and Cunningham 536

Pharmacology

65 Ephedrine A Review of More Recent Botanical Researches Alkaloidal Content of the Crude Drug and Experiments with Ephedrine and Pseudo ephedrine to Elucidate their Action by Dr Bernard E Read 537
Discussion—Kubota and Chopra 541
66 A Retrospect of Six Years Research Work on the Indian Indigenous Drugs by Lieut Col R N Chopra I M S 543
Discussion—Tomb Maya Das Subba Rao Basu (K P) Vyas and Chopra 551
67 Observations on the Stability of Chloride of Lime Stabilized Chloride of Lime and Perchloron in the Plains of Bengal by Dr J Walker Tomb O B E 553
Discussion—Banerjea (N L) Jansen and Tomb 567
68 Opium Habit in India by Lieut Col R N Chopra I M S and Dr Khem Singh Grewal 568
69 Study on the New Synthetic Analeptic Cardiazol by Dr A Kessler 576
Discussion—Chopra and Kessler 582
70 Some Notes on the Study of Chinese Drugs by Dr Seiko Kubota 583
Discussion—Chopra 586
71 On the Physiological Action of Anions by Dr N Onodera Dr E Nishio Dr M Yoshiki and Dr K Yukawa 587
Discussion—Chopra Sarkar (S L) and Onodera 590

SECTION VI

Veterinary

72 Animal Infectious Diseases and their Control in Japan by Dr Norichika Nakamura 592
Discussion—Edwards 596
73 Bovine Tuberculosis in India by Mr J T Edwards I V S 598
74 Virulence of Tubercle Bacilli Isolated from Cattle in India by Dr M B Soparkar 603
Discussion—Dey (D) Krishnamurti Ayyar, Nakamura Gharpure, Williams (A J) Soparkar and Edwards 624

		PAGE
75	An Improved Vaccine for Immunization against Rinderpest by Major R A Kelser v c Drs Stanton Youngberg and Teodulo Topacio	628
	Discussion —Edwards Hewlett Quirke and Vedder	645
76	The Treatment of Canine Piroplasmosis by Major R F Stirling I V S	647
	Discussion —Edwards, Krishnamurti Ayyar Ware, Williams (A J) Dey (D) and Edwards	651
77	On the Morphology of the Virus of Contagious Pleuropneumonia of Cattle (Demonstration) by Dr Tenji Taniguchi ..	651
	Discussion — Edwards	655
78	Generalized Infection of *Cœnurus serialis* or *Multiceps gaigeri* in Goats by Rai Saheb Debakar Dey I V S	656
79	Rhinosporidiosis in Cattle A Case recorded in a Bullock by Mr V Krishnamurti Ayyar	658
	Discussion —Edwards and Krishnamurti Ayyar	661
80	Gastro enteritis Hæmorrhagica in the Cattle of Formosan Milkers by Major Tosinobu Miyamoto Drs Tositune Nomura and Siwiti Ono	665
81	Urocystitis Hæmorrhagica of Native Cattle in Formosa by Major Tosinobu Miyamoto	667
	Discussion —Edwards Krishnamurti Ayyar and Miyamoto	681
82	Strongyloidosis Intestinalis in the Farrow of Formosa by Major Tosinobu Miyamoto	686
	Discussion —Ware	698
83	Rinderpest Some Properties of the Virus and Further Indications for its Employment in the Serum Simultaneous Method of Protective Inoculation by Mr J T Edwards, I V S	699
	Discussion —Krishnamurti Ayyar Williams (A J) and Edwards	705
84	Rinderpest Some Points in Immunity by Mr J T Edwards, I V S .	707
	Discussion —Williams (A J), Krishnamurti Ayyar, Ware and Edwards	715
85	Resolutions Passed at the General Business Meeting of the 7th Congress F E A T M .. .	718
86	EXHIBITIONS	724
	Scientific . .	725
	Commercial	7?0
	INDEX OF AUTHORS	755

LIST OF ILLUSTRATIONS

Article No		Facing Page
6	The Clasmatocyte in Experimental Kala azar Plates I and II	60 61
7	Studies upon the Peripheral Blood Bone marrow and Spleen of Hamsters experimentally infected with Kala azar Plate III	70
9	Erosion of the Inner Table of the Skull by Hyperplasia of Bone marrow in Kala azar with Extra medullary Formation of Blood on the Surface of the Dura Plates IV and V	85 86
13	Some Anopheles of Sarawak Plate VI	114
15	Parasitic Nematodes of *Anopheles* in Bengal Plate VII	132
16	Microsporidian Parasites of Anopheles Larvae Plate VIII	141
20	The Identification and Classification of the Species of the Genus *Phlebotomus* with some Remarks on their Geographical Distribution in Relation to Disease Plate IX	181
26	The Present Status of *Sauricola* and *Echinoplary x* Plate X	210
27	*Studies of Prophylaxis of Clonorchiasis* Plates XI XII XIII and XIV	213 214 215
31	*Filaria malayi* n sp parasitic in Man in the Malay Archipelago Plate XV	288
48	An Investigation to Determine a Satisfactory Standard for Beri beri Preventing Rices Plates XVI and XVII	408
64	The Action of Ether on the Rabies Virus Plate XVIII	532
65	Ephedrine A Review of More Recent Botanical Researches Alkaloidal Content of the Crude Drug and Experiments with Ephedrine and Pseudo ephedrine to Elucidate their Action Plates XIX and XX	540
69	Study on the New Synthetic Analeptic Cardiazol Plates XXI XXII XXIII XXIV XXV XXVI XXVII XXVIII and XXIX	580
77	On the Morphology of the Virus of Contagious Peripneumonia of Cattle Plate XXX	654
78	Generalized Infection of *Cœnurus serialis* or *Multiceps gaig r* in Goats Plates XXXI and XXXII	656
79	Rhinosporidiosis in Cattle Plates XXXIII and XXXIV	662 663
81	Urocystitis Hæmorrhagica of Native Cattle in Formosa Plates XXXV XXXVI XXXVII and XXXVIII	682

PAGE

75 An Improved Vaccine for Immunization against Rinderpest by Major R A Kelser v c Drs Stanton Youngberg and Teodulo Topacio 628
Discussion —Edwards Hewlett Quirke and Vedder 645

76 The Treatment of Canine Piroplasmosis by Major R F Stirling r v s 647
Discussion —Edwards Krishnamurti Ayyar Ware Williams (A J) Dey (D) and Edwards 651

77 On the Morphology of the Virus of Contagious Pleuropneumonia of Cattle (Demonstration) by Dr Tenji Taniguchi . 654
Discussion — Edwards 655

78 Generalized Infection of *Coenurus serialis* or *Multiceps gaigeri* in Goats by Rai Saheb Dibakar Dey r v s 656

79 *Rhinosporidiosis in Cattle A Case recorded in a Bullock by* Mr V Krishnamurti Ayyar 658
Discussion —Edwards and Krishnamurti Ayyar 664

80 Gastro enteritis Hæmorrhagica in the Cattle of Formosa in Milkers by Major Tosinobu Miyamoto Drs Tosituna Nomura and Siwiti Ono 665

81 Urocystitis Hæmorrhagica of Native Cattle in Formosa by Major Tosinobu Miyamoto 667
Discussion —Edwards Krishnamurti Ayyar and Miyamoto 684

82 Strongyloidosis Intestinalis in the Farrow of Formosa by Major Tosinobu Miyamoto 686
Discussion —Ware 698

83 Rinderpest Some Properties of the Virus and Further Indications for its Employment in the Serum Simultaneous Method of Protective Inoculation by Mr J T Edwards, r v s 699
Discussion —Krishnamurti Ayyar Williams (A J) and Edwards 705

84 Rinderpest Some Points in Immunity by Mr J T Edwards r v s 707
Discussion —Williams (A J) Krishnamurti Ayyar, Ware and Edwards 715

85 Resolutions Passed at the General Business Meeting of the 7th Congress F E A T M 718

86 EXHIBITIONS 724
- Scientific 725
Commercial 750

INDEX OF AUTHORS 755

LIST OF ILLUSTRATIONS

Article No		Facing Page
6	The Clasmatocyte in Experimental Kala azar, Plates I and II	60 61
7	Studies upon the Peripheral Blood, Bone marrow and Spleen of Hamsters experimentally infected with Kala azar, Plate III	76
9	Erosion of the Inner Table of the Skull by Hyperplasia of Bone marrow in Kala azar with Extra medullary Formation of Blood on the Surface of the Dura Plates IV and V	85 86
13	Some Anopheles of Sarawak, Plate VI	114
15	Parasitic Nematodes of *Anopheles* in Bengal Plate VII	132
16	Microsporidian Parasites of Anopheles Larvæ, Plate VIII	141
20	The Identification and Classification of the Species of the Genus *Phlebotomus*, with some Remarks on their Geographical Distribution in Relation to Disease, Plate IX	181
26	The Present Status of *Saurucola* and *Echinopharynx* Plate X	210
27	Studies of Prophylaxis of Clonorchiasis Plates XI, XII XIII and XIV	213 214, 215
34	*Filaria malayi* n sp, parasitic in Man in the Malay Archipelago Plate XV	288
48	An Investigation to Determine a Satisfactory Standard for Beri beri Preventing Rices Plates XVI and XVII	408
64	The Action of Ether on the Rabies Virus, Plate XVIII	532
65	Ephedrine A Review of More Recent Botanical Researches, Alkaloidal Content of the Crude Drug and Experiments with Ephedrine and Pseudo ephedrine to Elucidate their Action Plates XIX and XX	540
69	Study on the New Synthetic Analeptic Cardiazol Plates XXI, XXII XXIII XXIV, XXV, XXVI XXVII XXVIII and XXIX	580
77	On the Morphology of the Virus of Contagious Peri pneumonia of Cattle, Plate XXX	651
78	Generalized Infection of *Cœnurus serialis* or *Multiceps gaigeri* in Goats Plates XXXI and XXXII	656
79	Rhinosporidiosis in Cattle, Plates XXXIII and XXXIV	662, 663
81	Urocystitis Hæmorrhagica of Native Cattle in Formosa, Plates XXXV, XXXVI, XXXVII and XXXVIII	682

LIST OF SCIENTIFIC SECTIONS

VOLUME I

SECTION I

Medicine and Dermatology, Pathology, Surgery, Ophthalmology, Gynæcology and Diseases of Pregnancy, Mental Hygiene and Psychiatry, Radiology Dentistry

SECTION II

State Medicine, General and Special Hygiene, Maternity and Child Welfare

VOLUME II

SECTION III

Plague, Cholera, Dysentery, Sprue and Intestinal Infections, Bacteriophage, Leprosy, Tuberculosis, Bacteriology

SECTION IV

Typhus like Diseases and Leptospiræ, etc, Protozoology, Malaria (control) Malaria (general) and Malaria (treatment)

VOLUME III

SECTION IV—*Contd*

Kala azar, Medical Entomology Helminthology

SECTION V

Diseases of Nutrition, Deficiency Diseases, Immunology and Chemotherapy, Rabies and Anti rabic Treatment Pharmacology

SECTION VI

Veterinary
Scientific and Commercial Exhibitions.

CHAIRMEN AND RAPPORTEURS

SECTION IV—(*Continued*)

Kala azar

Thursday, 8th December, 10 A M to 1 P M	*Chairman*—Lieut Col S P James, I M S (*Retd*) (Great Britain)
	Rapporteur—Dr L E Napier (Bengal)
Saturday, 10th December, 10 A M to 11 45 A M	*Chairman*—Lieut Col S P James I M S (*Retd*) (Great Britain)
	Rapporteur—Dr L E Napier (Bengal)

Medical Entomology

Friday, 9th December, 10 A M to 1 P M	*Chairman*—Bt Col S R Christophers C I E, O B E F R S, K H P, I M S (B India)
	Rapporteur—Captain P J Barraud (B India)

Helminthology

Thursday, 8th December, 2 P M to 4 P M	*Chairman*—Col I Froilano de Mello (Portuguese India)
	Rapporteur—Dr V T Korke (B India)
Friday 9th December 10 A M to 1 P M	*Chairman*—Dr H Kobayashi (Japan)
	Rapporteur—Dr V T Korke (B India)
Friday, 9th December 2 P M to 4 P M	*Chairman*—Col S L Brug (Java)
	Rapporteur—Dr V T Korke (B India)

SECTION V

Nutrition

Monday, 5th December, 11 A M to 1 P M	*Chairman*—Col Edward B Vedder (Philippine Islands)
	Rapporteur—Major Clive Newcomb, I M S (Madras)
Monday, 5th December, 2 P M to 4 P M	*Chairman*—Col Edward B Vedder (Philippine Islands)
	Rapporteur—Major Clive Newcomb, I M S (Madras)

Deficiency Diseases

Tuesday 6th December, 10 A M to 1 P M — *Chairman*—Dr Victor G Heiser (U S A)
Rapporteur—Major J A Cruickshank, M C, I M S (*Retd*) (Madras)

Tuesday, 6th December, 2 P M to 4 P M — *Chairman*—Dr Victor G Heiser (U S A)
Rapporteur—Major Clive Newcomb I M S (Madras)

Immunology and Chemotherapy

Wednesday, 7th December, 10 A M to 1 P M. — *Chairman*—Professor S Hata (Japan)
Rapporteur—Captain K R K Iyengar, I M S (B India)

Wednesday, 7th December, 2 P M to 4 P M — *Chairman*—Dr Tenji Taniguchi (Japan)
Rapporteur—Captain K R K Iyengar, I M S (B India)

Rabies and Anti rabic Treatment

Pharmacology

Thursday 8th December 10 A M to 1 P M — *Chairman*—Dr B C P Jansen (Netherland Indies)
Rapporteur—Lieut Col. R N Chopra I M S (Bengal)

SECTION VI

Veterinary

Wednesday, 7th December, 10 A M to 1 P M — *Chairman*—Col A J Williams R A V C (B India)
Rapporteur—Mr F Ware I V S (Madras)

Thursday 8th December, 10 A.M to 1 P M — *Chairman*—Mr J T Edwards I V S (United Provinces)
Rapporteur—Mr F Ware, I V S (Madras)

SECTION IV.—(*Contd.*)

KALA-AZAR.

THE KALA AZAR TRANSMISSION PROBLEM AND THE FACTOR OF RESISTANCE

BY

LIEUT COL R KNOWLES I M S

Professor of Protozoology Calcutta School of Tropical Medicine

THURSDAY, DEC 8TH, 10 A.M TO 1 P.M

I FEEL it a very great honour to have been asked to open this discussion when there are so many other abler and more experienced workers than myself present My only excuse for occupying this position is that I have been associated with research work on this problem for the past ten years and my interest in it is still as keen as it was in 1917

I will not waste your time by referring to such exploded theories as that kala azar is transmitted by soil contamination or by the bed bug or *Triatoma (Conorhinus) rubrofasciata*, or that the leishmania parasites are in some mysterious way natural herpetomonads of various insects Instead let me try to sketch very briefly the history of the discovery that leishmania infections are almost certainly transmitted by sandflies

The earliest worker to draw attention to the sandfly in connection with oriental sore appears to have been Pressat (1905) as quoted by Grassi (1907) It is to be noted that the local name for oriental sore in Tashkent is *pasch i chu ly* which means fly bite As this can hardly refer to Stomoxys or the Tabanidæ it would appear to mean sandfly bite In 1905 the brothers Sergent attempted to produce oriental sores in man by the bites of sandflies collected at Biskra a hotbed of the disease, but failed probably because the men on whom they experimented were already infected or immune In 1911 Wenyon drew attention to this insect He records that of four patients who developed oriental sores in Bagdad three stated definitely that they had been bitten at the site of the sores by mosquitoes but the fourth incriminated a sandfly At this stage Wenyon appears to have almost guessed the truth, but to have been misled on to the track of the mosquito as *Aegypti* It is to be noted further that Wenyon (1911) found about 6 per cent of the sandflies of Aleppo infected with a herpetomonad This may well have been the herpetomonad phase of *Leishmania tropica* Mackie (1914) discovered

1

SECTION IV.—(*Contd.*)

KALA-AZAR.

THE KALA AZAR TRANSMISSION PROBLEM AND THE FACTOR OF RESISTANCE

BY

LIEUT COL. R. KNOWLES, I.M.S.

Professor of Protozoology, Calcutta School of Tropical Medicine

THURSDAY, DEC 8TH 10 A.M. TO 1 P.M.

I FEEL it a very great honour to have been asked to open this discussion when there are so many other abler and more experienced workers than myself present. My only excuse for occupying this position is that I have been associated with research work on this problem for the past ten years and my interest in it is still as keen as it was in 1917.

I will not waste your time by referring to such exploded theories as that kala-azar is transmitted by soil contamination or by the bed bug or *Triatoma (Conorhinus) rubrofasciata* or that the leishmania parasites are in some mysterious way natural herpetomonads of various insects. Instead let me try to sketch very briefly the history of the discovery that leishmania infections are almost certainly transmitted by sandflies.

The earliest worker to draw attention to the sandfly in connection with oriental sore appears to have been Pressat (1905) as quoted by Grassi (1907). It is to be noted that the local name for oriental sore in Tashkent is *pascha chundj*, which means fly bite. As this can hardly refer to Stomoxys or the Tabanidae, it would appear to mean sandfly bite. In 1905 the brothers Sergent attempted to induce oriental sores in man by the bites of sandflies collected at Biskra, a hotbed of the disease, but failed, probably because the men on whom they experimented were already infected or immune. In 1911 Wenyon drew attention to this insect. He records that of four patients who developed oriental sores in Bagdad three stated definitely that they had been bitten at the site of the sores by mosquitoes, whilst the fourth incriminated a sandfly. At this stage Wenyon appears to have almost guessed the truth, but to have been misled on to the track of the mosquito *Stegomyia aegypti*. It is to be noted further, that Wenyon (1911) found about 6 per cent of the sandflies of Aleppo infected with a herpetomonad. This may well have been the herpetomonad phase of *Leishmania tropica*. Mackie (1914) discovered

what he thought to be a herpetomonad—(but which Shortt 1925 claims to be a Bodo)—in the intestine of *Phlebotomus minutus* in Assam and drew special attention to the possibility of sandflies being the vectors of kala azar The Sergent brothers Lemaire and Senevet (1915) attempted to transmit oriental sore by the bite of *P minutus* but failed

In 1919 Acton drew attention to the fact that the distribution of sandfly bites and of oriental sores on the surface of the body are identical and his paper seems to have been *the first definite step forward In 1921 the brothers Sergent* Parrot Donatien and Beguet were the first workers to obtain positive results with the sandfly and produced an oriental sore on the arm of a volunteer by inoculation of an emulsion of sandflies captured in the wild state In S America Cerqueira (1920) recorded cases of espundia following at the sites of sandfly bites Aragao (1922) found herpetomonads in wild *P intermedius*, on inoculation of this material into a dog typical espundia followed with leishmania present in the lesions

In 1922 it was decided at an informal conference at the Calcutta School of Tropical Medicine that if necessary every other line of work should be suspended and all attention concentrated on the kala azar transmission problem The first question to be answered was What is the season of the year when infection with *kala azar is usually acquired ? Now the aldehyde test of Napier (1922) does not* become positive until the fifth month of the disease and a study of his cases led Napier to believe that the chief season of onset of kala azar lay in the monsoon

an analysis by Napier of all the cases which he had treated up to date showed that conditions were very different in north and in central Calcutta The imported cases of kala azar who had contracted the infection outside the city but had come into the city for treatment at the big hospitals, lived wherever they could find accommodation chiefly in north Calcutta On the other hand of the persons who *had been born* and bred in Calcutta itself and who had contracted kala azar in Calcutta some 60 per cent came from a very limited area in and around Ward 14 and Entally In other words although kala azar infection is being constantly imported into northern Calcutta for some reason it does not spread there on the other hand an endemic and spreading *focus of the disease exists in and around* Ward 14

This led to a very thorough and exhaustive survey of both areas from the epidemiological point of view and ultimately to the publication of Napier s two epidemiological studies of the disease (1925 1926) In the summary of his 1926 paper Napier records the following 21 conditions as associated with the spread of kala azar —

(1) An altitude of less than 2 000 feet above sea level
(2) A heavy annual rainfall in the region of or more than 50 inches
(3) Alluvial soil

(4) An equable humid tropical climate as indicated by —

(*a*) A monthly mean maximum temperature that is always below 100°F

(*b*) A monthly mean minimum temperature that is always above 45°F

(*c*) An annual diurnal range that is less than 20°F

(*d*) An annual mean relative humidity of at least 70 per cent

(*e*) A relative humidity for at least three months of the year which seldom falls below 80 per cent in the 24 hours

(5) A high subsoil water level which is maintained as such throughout the year

(6) Areas of abundant vegetation

(7) Rural districts in general rather than towns

(8) In particular old established villages sheltered by vegetation In towns the disease is associated with —

(9) Unprotected earth rather than paved courtyards and compounds

(10) Vegetation in close proximity to the dwelling

(11) Ground floor residences

(12) Thick walled masonry houses rather than thin walled bamboo and plaster huts

(13) Accumulations of refuse and untidy conditions in the compounds, especially when these are connected with the keeping of chickens and ducks

Further the transmitting vector must be one—

(14) Whose breeding ground is in some way connected with a definite site in or around a dwelling and most probably with the soil (as destruction of the dwelling still leaves the site infected)

(15) Which has a very short range of activity (less than 300 yards) and which if a flying insect has a very short range of flight and is very susceptible to air currents

(16) Which has a long life or is capable of maintaining an infection by transmitting it to the next generation

(17) Which does not readily establish itself on a new site but which under particularly favourable circumstances is capable of a steady extension of range

(18) Whose extension of range is checked by broad rivers by the sea by high ground (over 2 000 feet) and by unsuitable (non alluvial) soil

(19) Which does not ordinarily travel on man's person or in his belongings

(20) Which is freely associated with human beings of both sexes all ages races religions and castes but which on the whole is more freely associated with poorer class Anglo Indians Indian Christians and Mohammedans than with Hindus and with children of the second and third quinquennial age periods and finally

(21) Which is very prevalent possibly more prevalent during the monsoon months

Now there are practically speaking only two classes of biting insects which will fit in with the above outlined 21 conditions viz certain species of sandflies and the Culicoides

During the whole of 1922 and 1923 we attempted in vain to secure skilled entomological assistance but in July 1924 the Indian Research Fund Association very kindly placed at our disposal the services of Military Assistant Surgeon R O A Smith I M D and a special kala azar enquiry was financed at the School by that Association On a survey of the sandflies present in Calcutta only three species were found *P squamipleuris*—which however is present in such scanty numbers that it is very unlikely to be the vector — *P minutus* which appears to live entirely on the gecko and never, or hardly ever to bite man or other warm blooded host, and *P argentipes* Accordingly during the monsoon months of 1924 our efforts were concentrated on *P argentipes* Results exceeded even our happiest anticipations Between the 20th September and the 14th November 1924 in 10 out of 11 consecutive experiments female laboratory bred *P argentipes* fed upon the blood of kala azar patients showed typical herpetomonad forms in their midgut on the third to the fifth day after the infective feed and in six instances the infection was a heavy one though the patients' blood films had shown only very scanty parasites present In one instance two *out of three flies fed on a patient suspected to be suffering from kala azar showed* herpetomonads on the fourth day, it was only subsequently proved that the case was one of kala azar by spleen puncture Further the intensity of the infection present in some of the flies was almost incredible As controls to these experiments the gut contents of 811 other sandflies—including 107 *P argentipes* identified as such—and of 16 laboratory bred *P argentipes* fed upon persons suffering from diseases other than kala azar were examined not a single herpetomonad form being found Our results were embodied in a paper published in December 1924 (Knowles Napier and Smith, 1924) in which we concluded that *Leishmania donovani* passes into its herpetomonad phase in the midgut of the sandfly—*Phlebotomus argentipes*—under suitable atmospheric conditions of temperature and humidity

In the meantime the Kala azar Commission under the Indian Research Fund Association had been established *in Assam its members at first being* Col Christophers Major Shortt and Mr Barraud *later Major Shortt Mr Barraud* and Capt Craighead The Commission immediately took up our finding with regard

1925 (Christophers,

ealing with further

l was done by the

Commission By experimental evidence they ruled out of court such biting insects as the bed bug the Culicoides and *Triatoma rubrofasciata* They proved the whole cycle of development of *L donovani* in *P argentipes*, showed the successive forward invasion in turn of the œsophagus pharynx and buccal cavity and finally completed the whole account of the life cycle and morphology of the parasite in

P argentipes in a classical memoir by Shortt Barraud and Craighead (1926) Massive infection of the buccal cavity of the fly at the seventh to eighth day after the feed was demonstrated. Naturally infected *P argentipes* was found in connection with an outbreak at Pusa. The infectivity of the herpetomonad forms in the sandfly was experimentally proved. Extensive studies were made of the longevity of *P argentipes* under certain climatic conditions. The technique of re feeds was demonstrated. It was shown in a further important memoir that whereas in the case of the natural herpetomonads of insects transmission occurs in the posterior direction the infective forms being passed in the insect's fæces and infection being acquired by the larvæ swallowing them in the case of leishmania infections transmission occurs in the forward direction and is presumably contracted via the bite of the insect. In the meantime parallel work had been carried out by Adler and Theodor (1925—1927) on oriental sore in Palestine leading to the incrimination of *P papatasii* (or possibly of *P sergenti*) as the almost certain vector of that disease. Further in China Patton and Hindle (1927) had incriminated *P major* var *chinensis* as the vector of kala azar in that country.

In March 1926 the position appeared to be extremely favourable. By now it was practically certain that in India at least *P argentipes* is the natural vector of kala azar. One and only one further final proof was now necessary to actually transmit kala azar from man to man or to an experimental laboratory animal by the bite of infected *P argentipes*. The workers in Assam and those in Calcutta now concentrated all their efforts on giving this one, last convincing proof. Between March 1926 and September 1927 an enormous volume of work has been carried out infective flies being fed on mice hamsters monkeys and human volunteers. Yet despite the utmost efforts of the workers in both places these experiments have completely failed. When the Anopheline transmission of malaria was discovered actual transmission from man to man by infected Anopheles was readily secured but transmission of kala azar by *P argentipes* still awaits the final convincing proof.

What is the reason for this complete and exceedingly disappointing failure?

In the writer's belief the reason is that man—and still more so the experimental animal—so far from being readily susceptible to infection with kala azar is extraordinarily resistant to such infection. This may sound a most unorthodox view but the cumulative evidence of the last few years has forced this conviction on me. The Chinese hamster is said to be very susceptible to infection in China but in Calcutta at least we have found it not more susceptible than the white mouse possibly the difference is associated with the fact that the hamster hibernates *during the winter in China but does not do so in Calcutta*.

I will now try to marshal the evidence in favour of this thesis that man is relatively resistant to infection with kala azar.

(a) *Epidemiological evidence.* The mode of spread of epidemic kala azar is very different from that of epidemic malaria. When malaria is epidemic it is apt to be virulently so one has only to recall the great epidemic of 1908 in the Punjab

Now there are practically speaking only two classes of biting insects which will fit in with the above outlined 21 conditions viz certain species of sandflies and the Culicoides

During the whole of 1922 and 1923 we attempted in vain to secure skilled entomological assistance but in July 1924 the Indian Research Fund Association very kindly placed at our disposal the services of Military Assistant Surgeon R O A Smith I M D and a special kala azar enquiry was financed at the School by that Association On a survey of the sandflies present in Calcutta only three species were found *P squamipleuris*—which however is present in such scanty numbers that it is very unlikely to be the vector — *P minutus* which appears to live entirely on the gecko and never or hardly ever to bite man or other warm blooded host and *P argentipes* Accordingly during the monsoon months of 1924 our efforts were concentrated on *P argentipes* Results exceeded even our happiest anticipations Between the 20th September and the 14th November 1924 in 10 out of 11 consecutive experiments female laboratory bred *P argentipes* fed upon the blood of kala azar patients showed typical herpetomonad forms in their midgut on the third to the fifth day after the infective feed and in six instances the infection was a heavy one though the patients' blood films had shown only very scanty parasites present In one instance two out of three flies fed on a patient suspected to be suffering from kala azar showed herpetomonads on the fourth day it was only subsequently proved that the case was one of kala azar by spleen puncture Further the intensity of the infection present in some of the flies was almost incredible As controls to these experiments the gut contents of 811 other sandflies—including 407 *P argentipes* identified as such—and of 46 laboratory bred *P argentipes* fed upon persons suffering from diseases other than kala azar were examined not a single herpetomonad form being found Our results were embodied in a paper published in December 1924 (Knowles Napier and Smith 1924) in which we concluded that *Leishmania donovani* passes into its herpetomonad phase in the midgut of the sandfly—*Phlebotomus argentipes*—under suitable atmospheric conditions of temperature and humidity

In the meantime the Kala azar Commission under the Indian Research Fund Association had been established in Assam its members at first being Col Christophers Major Shortt and Mr Barraud later Major Shortt Mr Barraud and Capt Craighead The Commission immediately took up our finding with regard

… January 1925 (Christophers

… papers dealing with further

… work was done by the

Commission By experimental evidence they ruled out of court such biting insects as the bed bug the Culicoides and *Triatoma rubrofasciata* They proved the whole cycle of development of *L donovani* in *P argentipes* showed the successive forward invasion in turn of the œsophagus pharynx and buccal cavity and finally …mpleted the whole account of the life cycle and morphology of the parasite in

P argentipes in a classical memoir by Shortt Barraud and Craighead (1926) Massive infection of the buccal cavity of the fly at the seventh to eighth day after the feed was demonstrated Naturally infected *P argentipes* was found in connection with an outbreak at Pusa The infectivity of the herpetomonad forms in the sandfly was experimentally proved Extensive studies were made of the longevity of *P argentipes* under certain climatic conditions The technique of re feeds was demonstrated It was shown in a further important memoir that whereas in the case of the natural herpetomonads of insects transmission occurs in the posterior direction the infective forms being passed in the insect s fæces and infection being acquired by the larvæ swallowing them in the case of leishmania infections transmission occurs in the forward direction and is presumably contracted via the bite of the insect In the meantime parallel work had been carried out by Adler and Theodor (1925—1927) on oriental sore in Palestine leading to the incrimination of *P papatasii* (or possibly of *P sergenti*) as the almost certain vector of that disease Further in China Patton and Hindle (1927) had incriminated *P major var chinensis* as the vector of kala azar in that country

In March 1926 the position appeared to be extremely favourable By now it was practically certain that in India at least *P argentipes* is the natural vector of kala azar One and only one further final proof was now necessary to actually transmit kala azar from man to man or to an experimental laboratory animal by *the bite of infected P argentipes The workers in Assam and those in Calcutta now* concentrated all their efforts on giving this one last convincing proof Between March 1926 and September 1927 an enormous volume of work has been carried out infective flies being fed on mice hamsters monkeys and human volunteers Yet despite the utmost efforts of the workers in both places these experiments have completely failed When the Anopheline transmission of malaria was discovered actual transmission from man to man by infected Anopheles was readily secured but transmission of kala azar by *P argentipes* still awaits the final convincing proof

What is the reason for this complete and exceedingly disappointing failure ?

In the writer s belief the reason is that man—and still more so the experimental animal—so far from being readily susceptible to infection with kala azar is extraordinarily resistant to such infection This may sound a most unorthodox view but the cumulative evidence of the last few years has forced this conviction on me The Chinese hamster is said to be very susceptible to infection in China but in Calcutta at least we have found it not more susceptible than the white mouse possibly the difference is associated with the fact that the hamster *hibernates during the winter in China but does not do so in Calcutta*

I will now try to marshal the evidence in favour of this thesis that man is relatively resistant to infection with kala azar

(*a*) *Epidemiological evidence* The mode of spread of epidemic kala azar is very different from that of epidemic malaria When malaria is epidemic it is apt to be virulently so one has only to recall the great epidemic of 1908 in the Punjab

when over a million persons died of malaria and *sporozoite rates of from 10 to 18* per cent were recorded in the transmitting Anophelines Epidemic kala azar on the other hand spreads slowly but irresistibly, often taking some years to spread a few miles As pointed out by McCombie Young (1924) the great Nowgong *epidemic followed upon the Assam earthquake* when the economic condition of the people was seriously reduced whilst the recrudescence of kala azar in Assam in 1919 onwards followed close upon the heels of the influenza pandemic of 1918 when the vitality of the population was seriously lowered Epidemic disease of any sort usually falls most heavily upon a debilitated population but there seems to be a specially close relationship between epidemic kala azar and conditions of enonomic distress or physical debility

(*b*) *Clinical evidence* —The majority of kala azar patients when first seen are suffering from kala azar with *L donovani* present in their tissues but this is by no means always the case At Calcutta we have been especially struck by the great frequency with which a secondary infection lights up the onset of kala azar Patient after patient has been admitted to our hospital suffering from typhoid fever with the *B typhosus* isolated from his blood stream or a strongly positive Widal reaction has recovered then has had what was apparently a relapse but on blood culture has shown *L donovani* present in the *blood* Case *after* case of true kala azar commences as a true malaria with malaria parasites present in the blood films at first the fever is amenable to quinine treatment and is subdued thereafter however it ceases to be amenable to quinine and on blood culture *L donovani* is isolated

It would appear in fact that the occurrence of some disease which reduces the patient s resistance leads to the onset of kala azar It is to be noted that both typhoid fever and malaria are usually associated with leucopenia Malaria is rather apt to be a disease of sthenic individuals it is the man in the most robust state of health who gets the worst rigor whereas kala azar is essentially a disease of asthenic individuals

Further there is considerable evidence of the existence of a condition of hypo adrenia in kala azar As its name implies kala azar is associated with an *increased pigmentation of the face* and such *increased* pigmentation is usually associated with hypo adrenia Col Acton informs me that amongst the thousands *of out patients whom he sees annually in the skin out patient* clinic at the School he has never yet seen the co existence of leucoderma with kala azar and *leucoderma is essentially associated with hyper adrenia*

Still again we have the curious disease to which attention was first called by Brahmachari (1922) dermal leishmanoid This has been especially studied by Acton and Napier (1927) In this condition the patient has either had kala azar has been treated and recovered *or else has never had* kala azar but comes from an endemic area He is in perfect health with a normal blood picture and culture of the peripheral blood fails to yield leishmania But tiny white leucodermic spots appear in the skin all over the body and gradually soft granulomatous nodules

appear, the final condition looking like a case of nodular leprosy nodules L donovani is readily recovered either in films or cultures (1927) has shown that such strains are capable of producing visceral intraperitoneal injection into mice Col Acton finds that there is hyper adrenia always associated with these cases of dermal leishm instance of three members of an Anglo Indian family living in the Calcutta the mother and daughter contracted kala azar but the dermal leishmanoid and not kala azar

In the condition of dermal leishmanoid it would appear that powers of resistance are such that L donovani is incapable of prod infection Yet the patient becomes infected with the parasite w the fine capillary plexuses underlying the skin and there produces The infection in these cases must be of an embolic character a within

(c) *Experimental evidence* —The uncertainty and extreme difficu kala azar is transmitted to experimental animals is very remarkabl massive doses of virus are injected the infection usually fails to take is injected with kala azar virus one of four things may happen —

(1) Usually the animal remains well and fit and on repeated carried out about once a month parasites may never be recovered films or cultures Thus of 23 monkeys 11 dogs 20 white rats and of which I have records in Calcutta 19 monkeys 9 dogs all 20 whi mice failed to show any infection at all or in all 61 out of 68 ani rate of 90 per cent

(2) Secondly if the injection be given intradermally a locali follow

(3) Thirdly a condition of transient leishmaniasis may f animal remains in good health and without symptoms but at a week liver puncture shows the presence of parasites either in fil Later on from about the ninth week onwards the results o punctures are negative in both films and cultures Five of the abo viz four monkeys and one pup showed this condition

(4) Lastly and exceptionally the animal may develop ac kala azar This occurred in one monkey and one pup of the above less than " per cent

It must be admitted that Shortt (1923) has had better results for he records that of 13 monkeys inoculated ten became infected them acute and fatal kala azar developed Shortt and Swaminat record successful infection of a monkey by the intradermal inj a c c of spleen juice Even under the most favourable conditi not more than about 10 to 15 per cent of inoculated ani

Taking the whole of this evidence together it seems to me that it is possible that in the endemic areas mankind in general may be inoculated wholesale with *Leishmania donovani* In the vast majority of such persons the parasite may never cause any symptoms at all There are several remarkable cases in the literature where infection with *L donovani* apparently lay latent for some years in one case as far as I remember the patient developed kala azar in New York five years after leaving the infected areas in Madras With regard to direct inoculation of man the only recorded experiments that I know of are those of Maggiore (1925) who inoculated four men with bone marrow from a case of kala azar three men with cultures of *L donovani* and three with cultures of *L tropica* In none of these *instances did infection follow*

Should the patient s resistance be high and should he be in a condition of hyper adrenia the parasites are incapable of causing a visceral infection but the condition of *dermal leishmanoid may follow In relatively few persons and* those usually who are in a condition of hypo adrenia or whose resistance is unduly lowered by high food prices or intercurrent disease especially typhoid and malaria the parasites are able to cause visceralized disease and kala azar results

If this hypothesis is true then it appears to me useless at this juncture to carry out further sandfly feeding experiments It is of course possible that some experimental animal or human volunteer may happen to be in a suitable condition of lowered resistance but even if one positive result is obtained it is likely that a second positive will not be obtained on repeating the experiment The lepers at the Purulia asylum on whom infected sandflies from Calcutta have been fed are probably too well looked after and in too robust a state of general health to contract infection The Khasis upon whom the Commission have fed infected sandflies if they are like any Khasis that I ever met are probably in a *condition of riotously good health I believe that the next step forward must* be to make a full study of this factor of resistance and of the causes underlying it and find out how to break it down

Certain experiments have been carried out at the School during recent years in an attempt to break down this resistance in the experimental animal Six monkeys were submitted to sub total thyroidectomy a quarter of each lobe of the gland being left behind in order to support life An interval of 15 days was then allowed *for the thyroxin* in their tissues to become burnt up and they were then inoculated with flagellate culture of *L donovani* two intraperitoneally and two intravenously and the remaining two intraperitoneally with fresh splenic emulsion The first four all failed to take the fifth monkey died *from some cause* not ascertained on the 12th day after injection and its viscera showed leishmania, the last monkey showed transient leishmaniasis subsequently The virus from the fifth animal was passaged *into two further thyroidectomized* animals and both showed transient leishmaniasis It would seem then that sub total thyroidectomy by lowering the general resistance of the animal may render it somewhat more *susceptible* but not markedly so In the second place injections of benzyl

benzoate were given to monkeys in the hope of inducing leucopænia in these animals, the injections were badly tolerated, however, and remained as unabsorbed lumps under the skin, whilst no marked leucopænia resulted. At present we are trying to knock out the adrenal activity of the experimental animals upon which sandflies are fed by carrying out a previous laparotomy and injecting a drop of pure carbolic acid into the adrenal gland on each side.

In conclusion I would state my belief that before the last and final proof that kala azar is transmitted by sandflies is afforded this factor of resistance will probably have to be studied and overcome.

REFERENCES

ACTON, H. W. (1919). A study of the distribution of Bagdad boils on the body with a view to discover the transmitting agent. *Ind Jour Med Res*, Vol VI, p 262.

ACTON, H. W., and NAPIER, L. E. (1927). Post kala azar dermal leishmaniasis. *Ibid*, Vol XV, p 97.

ADLER, S., and THEODOR, O. (1925). The experimental transmission of cutaneous leishmaniasis to man from *Phlebotomus papatasii*. *Ann Trop Med and Parasit*, Vol XIX, p 365.

Idem (1926). Further observations on the transmission of cutaneous leishmaniasis to man from *Phlebotomus papatasii*. *Ibid*, Vol XX, p 175.

Idem (1926a). On the identity of *Leishmania tropica* Wright 1903 and *Herpetomonas papatasi* Adler 1925. *Ibid*, Vol XX, p 355.

ARAGAO, H. DE B. (1922). Transmissão da leishmaniose no Brazil pelo *Phlebotomus intermedius*. *Brazil Medico*, Year 36, Vol I, p [illegible].

BRAHMACHARI, U. N. (1922). A new form of cutaneous leishmaniasis—dermal leishmanoid. *Ind Med Gaz*, Vol LVII, p 125.

CERQUEIRA, A. DE C. (1919). Contribuição ao estudo da patogenia da Leishmaniose americana. Nota prelim. Papel do *Phlebotomus* como transmissor da Leishmaniose Tegumentar. *Saúde*, Rio de Janeiro, Vol II, p 22.

CHRISTOPHERS, S. P., SHORTT, H. E., and BARRAUD, P. J. (1925). The development of the parasite of Indian kala azar in the sandfly *Phlebotomus argentipes* Annandale and Brunetti. *Ind Jour Med Res*, Vol XII, p 605, and *Ind Med Res Memoir*, No 4, p 123.

Idem (1925a). Further observations on the feeding of sandflies *Phlebotomus argentipes* on cases of Indian kala azar. *Ibid*, Vol XIII, p 159 and *Ind Med Res Memoir*, No 4, p 127.

Idem (1925b). *Culicoides* and kala azar in Assam. *Ibid*, p [illegible] and *Ind Med Res Memoir*, No 4, p 111.

Idem (1926). Development of the parasite of Indian kala azar [illegible] sandfly *Phlebotomus argentipes* controls. *Ind Med Res Memoir*, No 4, p 135.

Idem (1926a). Development of the parasite of Indian kala azar [illegible] sandfly *Phlebotomus argentipes* [illegible] and further results of the feeding of sandflies [illegible] cases. *Ibid*, p 141.

GRAS I L (1J07)	R cher l e su Flebotom *Memor e d lla Soc et Ital ana della S ien e* Ser 3a XII
GUPTA B M DAS (19 7)	A note on the para te of dern al le shmano d *Ind Med Ca* V l LXII p 11
Il (19 a)	I rther b er at ns pon dern al le hmano l *Il l* p 10
KNOWLES R (19?4	I eport of the I rofe or of I rotozoology *I n P pt Ca ut a S hool T op M d fo* 19 3 p 64
Idem (19)	Report of the I rofes or of I rotozoology *Ib d* for 19 4 p 71
Idem (19)	I eport of the I rofes or of Protozool gy *Ib l* for 19 6 p 53
Idem w tl G PTA B M DAS (19?4	On trans ent nfect ons w tl *I shman don an* n man an l an mals *Ind M d Ca* Vol LIX p ?9
KNO LES R NAPIER L E and GUPTA B M DAS (19 3)	The kala azar t an m on problem *Ib d* V l LVIII p 3 1
KNOWLES R NAPIER L E a d S T R O A (19 4)	On a *H rp to nona* found n tle gut of the sandfly *Phl o n s argent pe* fed on kala azar pat ents A prel n ary note *Ib d* Vol LIX p 593 and *I d M d Res M no* No 4 p 113
MACKIE F P (1914)	A fla ellate nfect on of sandfl es *Ind Jo r Med Res* Vol II p 3
Idem (1915)	Insects an l ka a azar *Ib d* p 94
MAGGIORE S (19 5)	Contr l uto allo stu l o et o patogenet co delle inf z on da Le sl man *P l at ca* Vol XXXIII p 169
NAPIER L E (19	A ne s rum test for kala azar *Ind Jour Med R s* Vol IX p 830
Id (19?5)	A con parat study of tle env ro n ent assoc ted w tl kala azar prevalence n Calcutta *Ib l* Vol XII p 755
Idem (19 6)	An ep l n olo al cons lerat on of tle tran n iss on of kala azar n Ind a *Ind Med Res Memo r* No 4 p ?19
NAPIER L E and S T R O A (19 6)	Furtl er ob er at ons on tle feed ng of sandfl es *Phl bolom s argent pe* on ca es of kal azar n Calcutta *Ib l* p 147
Idem (19 6a)	Tl e development of *Le sh n n a do o an* n tl e gut of tl e sandfly *Phlebo o us argent p s Ind Jour Med R s* Vol XIV p 713
PATTON W S and HINDLE E (19 7)	The development of Ch nese Le shman a n *Phl botomus* *m jo va cl ne s s* and *P se g nt* var *P oc Royal Soc* Vol CI Ser B p 369
SERGENT ED and ET LEMAIRE C and SENEVET G (1914)	Inse te t ansmetteur et réservo r de v rus du lou d B skra Hypothèse et xp r ences prél n na es *B ll Soc Path Exo* Vol VII p 577
Idem (1915)	Hypothèse sur le phlebotome transmetteur t tarante and r ervo r d v us du bouton d Or ent *Ann Inst Pas eu* Vol XXIX p 309
SERGENT ED and ET PARROT L DONATIEN A and BEGUET M (19 1)	Tran m s on lo clou de Biskra par le phleb tome (*Phlebo o nus papatas* S op) *C R Acad S* Vol CLXXIII p 1030
SHORTT H E (19 3)	Reco d of kala azar re ear h work c rried out at the I n Edwar l VII Men o ial Pa teur I st tute Sh llong d ng 19 *Ind Jour Med Res* Vol X p 1150

SHORTT H E (19 3) — The patholo y of acute exper m ntal kala azar n monkeys *Ib d* Vol XI p 186

Idem (19) — Note on *Bodo phlebotomi (Herpeto onas phl bo o n)* (Macki 1914) a recon deration of ts re er e pos t n *Ib I* Vol XIII p 141 a 1 *I d M l R s M mo* N 4 p

SHORTT H E BARRAUD P J and CRAIGHEAD A C (19 6) — Note on a m s e nf n of tl pharynx f *Phlebotomus argentipes* tl *Herp ton on d no* p 441 and *Ind M d Res Me o r* No 4 p 157

Idem (19 6a) — An acco nt of tl e methods employed n fee l and re feed ng san lfl es *Phlebotomus argentipes* for the second an l thi l times on man an l an m al *Ib I* p 9 3

Idem (19 6b) — The l fe h tory an l morphology of *H rp t u dono an* n the sandfly *Phl otom s a gent pes* *Ib I* p 947

Idem (19 6c) — *Cono h nu r fasc atus* d Greer a d Ind an kal azar *Ib I* Vol XIV p 39

Idem (19 6d) — Note on a ma e nfect on of the bu al av t f *Phlebotomus argentipes* w th *H peto on dono an* p 3

Idem (19 6 19) — Note on the infect ty of the form s of *Le h man a dono an* f d n *Phlebot m s a g nt p s I l* p 5 7

Idem (19 7a) — Tran m on exper ments n Indian kala azar th *Phlebotomus argentipes Ib d* p 589

SHORTT H E and SWAMINATH C S (19 5) — Experiments to dec de whether the be l b g *C ex h pt a* Fabr can transm t In l n kal azar *Ib I* Vol XIII p 143 and *Ind Med R s M o r* N 4 p 1

Idem (19 5a) — S tem nfe t n of a monk y (*Ma a s rhesus*) b ntr l rmal noculat on of spl en pun ture mater al from a case of In l an kala azar *Ib I* p 149 an l *Ind M d I Memo r* No 4 p 67

WENYON C M (1911) — Note th o c rrence of *Herpetomonas* n the *Phl o to* of Al ppo *Jou Lon I ool Trop M l* Vol I p 98

Idem (1911a) — Or ntal sore n Pa l d together w th obser at ons on a gr g r n n *Stegomyia f sc ta* tl Hamogregar ne of loc s an l the flagellates of hou e fl e *P s t lo y* Vo IV p 3

YOUNG T C McC (19 3) — The sea on of set of kala azar *Ind Med G* Vol LVIII p 5

Idem (19 4) — Kal azar n As am An account of the preventive operat on 1910 to 1 5 an l notes on the ep l m o lo y of the d sease n Ind a London

Idem (19 4a) — The kala azar tran m on problem (Correspon l nce) *Ind Med Ga* Vol LIX p 9

THE LIFE HISTORY OF *LEISHMANIA DONOVANI* IN ITS INSECT AND MAMMALIAN HOSTS

BY

MAJOR H. E. SHORTT, F.Z.S., I.M.S.

Director, Kala-azar Commission

In considering the life history of a protozoan which spends one cycle of its existence as an active flagellate parasite in an insect host and another cycle as an aflagellate immobile parasite in a mammalian host, we are dealing for all practical purposes with two separate life histories which are mutually connected at one point only. This point is the act of biting of the insect host in the process of which the parasite may on the one hand be acquired by the insect from the mammal or on the other hand be transmitted to the latter. In the case of *Leishmania donovani* except at the point where the two cycles in the life history meet, i.e. the bite of the insect, during no phase of the life cycle in the insect habitat does the organism appear to have any really close resemblance to the forms present in the mammalian habitat such as occurs in the case of the trypanosomes, and the parasites in the insect and mammalian hosts might be two separate and entirely disconnected species. Besides this morphological dissimilarity in the two phases of the complete life history there are other biological differences equally marked. Thus the (?) cycle in the insect occupies at most a week or two, that in the mammal may last for years. The cycle in the insect requires a comparatively low temperature not exceeding about 31°C, while that in the mammal may withstand a continuous temperature of well over 37°C. The cycle in the insect is a phase of great individual activity on the part of the parasites and these are pleomorphic. The cycle in the mammal is a phase of immotility on the part of the parasites and these are monomorphic.

I shall now proceed to deal with each cycle in the life history separately and then pass to a consideration of the only point common to each, viz., the act of biting by the fly.

LIFE CYCLE OF *Leishmania donovani* IN ITS INSECT HOST *Phlebotomus argentipes*

It has already been shown by the Kala-azar Commission that the optimum temperature for rapid multiplication of *L. donovani* in the sandfly is one of 28°C and that the maintenance of this temperature within very narrow limits of variation is essential for the completion of the life history of the flagellate in the fly

The reason for this is that the life history in the insect is not completed before a minimum of seven or eight days and for the insect to survive for this length of time it is necessary for it to oviposit and have a second feed The successful performance of oviposition is only attained when the temperature remains constantly in the neighbourhood of 28°C By keeping this principle in mind we have been able to feed flies up to a maximum of ten times oviposition occurring antecedent to each feed It must be clearly understood therefore that the account of the life history to be now given refers to the sequence of events occurring when the insect hosts are kept continuously at a uniform temperature of about 28°C

At its feed on a kala azar case *Phlebotomus argentipes* takes up a sufficiency of blood to ensure the presence of Leishman Donovan bodies in a large percentage of flies If such a fed fly be dissected after the elapse of twenty four hours the blood meal will be found to be compacted into a mass held together by what has the appearance of a delicate enclosing membrane If this be ruptured the blood is allowed to escape and it will be seen that digestive processes have started although much of the blood is still apparently unaltered No parasites will be visible in the fresh preparation

If a smear of the material be made and stained by any Romanowsky method a careful search will reveal a few *Leishmania* These are usually single but small groups may also be seen They are of two types (*a*) unaltered Leishman Donovan bodies (type 1 in the classification of Christophers Shortt and Barraud) (*b*) early flagellation forms (type 2) In the case of the latter it will be seen that the body of the parasite has increased markedly in size the flagellar vacuole has become very evident and stains a pink colour The cytoplasm stains an opaque china blue and has numerous vacuoles The flagellum is unformed or a rudiment only Division stages may be present and represent multiplication before attainment of the flagellate condition The parasites present are all absolutely immobile

Forty eight hours after the initial feed the appearances in a fresh preparation are still undistinctive A painstaking search may reveal the presence of a few sluggishly motile forms but these are not yet capable of swimming freely

In a stained preparation made at this stage two types of parasite again predominate These are —

(*a*) Stumpy or rounded flagellates with division forms of the same (type 3)

(*b*) Immature flagellates or larval forms (type 4)

Type 3 is comparatively very broad and is the form in which division occurs Its trophonucleus is relatively large and it has a well developed flagellum Type 4 is the first definitely elongate form to appear Its body is not markedly different in shape at either extremity and it possesses a well developed flagellum In spite of the latter structure type 4 at this stage shows practically no power of translatory movement although the flagellum is in continuous undulatory motion

Three days after the initial feed it is at once evident even in fresh preparations that a distinct advance in the development of the flagellate infection has taken

place This development is characterized by the appearance of numerous elongated free swimming flagellates In addition to these new forms there is an increase in the numbers of the flagellates but non free swimming forms already noted i e, forms 3 and 4 Division forms of type 3 are numerous indicating active multiplication and for the first time are seen small rosette formations the beginnings of the huge compound rosette agglomerations to be later mentioned The long free swimming flagellates are the mature flagellates (type 5) These elongate forms give one the impression of an intense and restless activity 'capable of carrying them into any situation penetrable by bodies of their bulk' At this stage the flagellate infection is more or less diffused throughout the midgut and the marked anterior concentration seen at a later stage is not yet much in evidence At the stage just described which is that reached by the third day after the initial feed there are present practically all the forms of flagellate to be seen at any later stage

From this period onwards the life cycle in the fly takes the form of intense reproductive activity resulting in a rapidly increasing number of all the forms already described until the anterior portion of the midgut becomes the scene of an impressive writhing activity indicative of the enormous concentration of flagellates in this situation This stage will have been reached by the fourth or fifth day at about the time that the fly oviposits for the first time and takes its second blood meal

At this stage it will be convenient to say something of the disposition of the flagellate infection with regard to the alimentary canal of the insect and of the relative numbers and special locations of the various types of flagellate already mentioned The description to be given refers to what would be seen if the entire alimentary canal of the insect were dissected out and the soft tissues of the head removed to expose the portion running through that region If the preparation be examined at once the walls of the alimentary canal have a rugose appearance which to some extent obscures a view of the contents but in spite of this a heavily infected fly shows a condition of seething motility which is very impressive This obvious motility is almost entirely due to the elongated flagellates either free swimming or loosely attached to their surroundings by their flagella, and gives no indication of the equally numerous or even more numerous sessile or semi sessile forms which either line the wall of the anterior part of the midgut or form densely packed masses in the proventricular region These latter forms are too tightly packed for their units to be capable of individual movement and only those individuals composing the periphery of the masses can take any part in producing the seething motility described If the preparation be covered with a cover slip the sessile forms become more evident It will now be seen that the flagellates are almost entirely concentrated in the anterior portion of the midgut the greatest massing being in the region of the proventricular fold The midgut here forms a distinct shoulder like a bottle the neck of the bottle representing the anterior portions of the alimentary canal from the œsophagus onwards In and about this fold the flagellates are densely packed in palisade formation many layers deep

sometimes almost blocking the lumen of the canal The flagellates composing this massive growth have a general antero posterior orientation and are composed chiefly of types 3 and 4 attached to the walls of the gut by their flagella or with the latter insinuated between the deeper layers of flagellates The massive growth of flagellates may, by the fifth day have invaded the posterior region of the pharynx and the commencement of the diverticulum More posteriorly the massive growth gradually thins out and this is accompanied by a change in the type of parasite which, with increasing freedom of movement tends to assume a more elongate form so that type 5 is here predominant In some cases there is a large development of rosette formations in the lumen of the midgut These are composed of agglomerations of elongate flagellates chiefly of type 5 and by the coalition of numerous rosettes huge compound masses may be formed which eventually may extend over half the width of the midgut at its widest part

If we come to consider the relative numbers of the different types of flagellates in an infection such as that described we find that certain types have a selective affinity for certain situations in the gut If the midgut be opened and the contents allowed to flow out without undue pressure they will represent the flagellate content of the lumen as opposed to the flagellates attached to the gut wall Preparations made from these two situations show entirely different characteristics These characteristics may be summarized by saying that the forms encountered in *preparations made from the lumen are elongate free swimming forms with types* 5 and 4 predominating, while preparations made from the gut wall show chiefly sessile or semi sessile forms with types 3 and 4 predominating

To return now to the continuation of the life history which we interrupted at the stage reached by the fifth day after the initial feed The fly has oviposited and had a second meal of blood This meal appears to have no deleterious effect on the continued development of the flagellates and the anterior progression of the massive growth of *L donovani* proceeds without interruption The great development of parasitic bodies dilates the extensible œsophagus and the wide posterior portion of the pharynx is rapidly filled with an extensive growth The anterior end of the flagellate mass in its growth forward is usually preceded by a certain number of free or attached elongate flagellates As the pharynx narrows anteriorly the column of flagellates also narrows although it fills the pharynx to the limits of its extensibility The junction of the pharynx with the buccal cavity is marked by a sharp angle in the alimentary canal and this situation may be reached by the massive parasitic growth by the seventh day after the original feed of the fly The narrow buccal cavity is now entered and the growth reaches its anterior end by the eighth to tenth day after the initial feed At this stage the most anteriorly placed flagellates may be protruding from the mouth proper of the fly into the prestomum This stage seems to mark the limit of development reached in the insect

A word should now be said with regard to the types of flagellate seen in the anterior portions of the infection from the œsophagus forwards The growth in its more massive parts where the canal is widest may be said to be composed chiefly

of types 3 and 4 i e of semi sessile forms Where the alimentary canal is most constricted i e in the anterior part of the pharynx and in the buccal cavity the flagellates tend to assume the elongate form being orientated antero posteriorly and represent chiefly type 5 (c) i e the small attenuated form of mature flagellate This also is the type represented in the most anterior portion of the growth which projects into the prestomum In smear preparations from the massive portions of the growth from any situation but especially from the proventricular region where the infection reaches its largest dimensions a certain number of aflagellate Row s bodies may be observed These are not believed to have any significance and have not been observed in the anterior regions of the mouth parts

LIFE CYCLE OF *Leishmania donovani* IN ITS MAMMALIAN HOST

If a fly infected to the extent of having a massive growth of flagellates in the buccal cavity should bite man it seems inevitable that a portion at least of the flagellate growth should be ejected into the wound caused by the bite As the piercing organs of the fly are arranged to form a food canal it follows that anything entering or leaving the proboscis must do so near its distal extremity As a consequence of this any flagellates ejected must be delivered into the tissues of the mammalian host at the depth to which the piercing organs penetrate As these are inserted to more than half their total length it follows that the inoculation of a mammal with flagellates occurs at a depth of about 170 μ in its tissues The thickness of the epidermal layer on the arm of man, although variable is such that the inoculated flagellates would find themselves transplanted into the vascular sub epidermal connective tissue of the mammal or even deeper into the subcutaneous tissues

Presuming then that such a transference of flagellates takes place the sequence of events following the bite of the fly may be described as follows —

(1) The flagellates ejected into the wound are engulfed probably singly but sometimes possibly in small groups by endothelial cells derived from the blood capillaries or lymph channels thus gaining an intracellular habitat

(2) In the process of becoming an intracellular parasite the organism loses its flagellum which drops off at the point where it leaves the periphery of the flagel late body The body of the parasite during this process rounds up until it assumes the form of the typical Leishman Donovan body with its trophonucleus parabasal flagellar vacuole and axoneme The time required for the conversion of a free flagellate into a fully formed intracellular aflagellate Leishman Donovan body probably varies under varying *conditions but the process can certainly* be completed in about three and a half hours

(3) The endothelial cell with its contained parasite now gains the blood *stream either directly* or through the lymph channels and may be carried to any part of the body but the sites of election for its arrest and the further development of the infection are probably the spleen liver and bone marrow in the order given

(4) The intracellular Leishman Donovan body now divides by binary fissi into two equal individuals

(5) Multiplication proceeds with the production of four, eight sixteen or mo individuals up to the capacity of the invaded cell to contain its load of parasites

(6) A point is finally reached where the endothelial cell becomes a mere sh and finally ruptures releasing the contained Leishman Donovan bodies

(7) These released parasites are taken up afresh by other endothelial cells the tissue in which they are lying and the process is repeated indefinitely un finally an intense infection of the endothelial tissues of the whole organ may resu accompanied by a great increase in size of the organ

(8) Instead of being taken up by the endothelial cells of the organ in whi they are released by the rupture of their parent cell some of the Leishman Donov bodies may be released into situations such as the blood sinuses of the spleen whe they can be taken up by enothelial cells or large mononuclear cells of the blo capillaries, thus giving rise to the presence of parasites in the peripheral blood of t mammal

So far as I have been able to judge the mammalian cell which is the host the parasite throughout the body is always an endothelial cell of very primitive an undifferentiated type Certain workers have reported the presence of parasites other cells, e g , in the proper cells of the liver This is an observation I have be unable to confirm In sections of the liver the apparent presence of parasites in t parenchyma cells is nearly always due to a misinterpretation of the orientation the parts in a preparation Moreover, in smear preparations, where there is chance of mistaking any other kind of cell for a liver cell proper I have never se the latter containing parasites

The Act of Biting of the Fly

In another publication by the writer and Swaminath it has been shown th when a very heavily infected fly feeds the most probable method for t transference of the infection to the mammal is by the injection into the wound of plug of flagellates rather than by the active invasion of the wound by sing free swimming flagellates In other words, the entry of flagellates into t wound is not an active process on the part of the flagellates involving any volitio on their part but a passive process caused by an expulsive action on the part the fly In any very heavily infected fly the part of the alimentary canal passi through the head is practically blocked by flagellates This blockage, especial in the narrowest portions which admit but a few red blood cells abreast wou prevent the entry of any considerable number of solid red blood cells Th conclusion drawn from this is that in any very heavily infected fly the expulsio of at least the more anteriorly placed flagellates and those occupying the narrowe parts of the food channel must precede the entry of a blood meal Th expulsion of flagellates must take place in an anterior direction, and therefore int the wound on account of the fact that the bulk of the flagellate growth . . .

greater in proportion as it extends backwards and therefore pressure applied to the walls of the food channel by muscular action at any point would inevitably tend to expel the contained flagellates in an anterior direction which would be the direction of least resistance

The general conclusion drawn is that if kala azar is transmitted by the bite of the sandfly, the infective material probably takes the form of a solid plug of flagel lates and this plug is introduced into the wound by an active expulsive effort by the sandfly preliminary to its taking a blood meal

KALA AZAR STUDIES IN NORTH CHINA

BY

CHARLES W YOUNG B SC, M D

AND

MARSHALL HERTIG Ph D

Department of Medicine Peking Union Medical College Peking, China *

THE following is a brief summary of the activities of the Kala azar Field Studies Unit of the Peking Union Medical College Peking China during the four years 1924 to 1927

The unit occupied as field headquarters a part of the hospital of the Southern Presbyterian Mission at Hsuchowfu Kiangsu Province This location was chosen on account of the active kala azar clinic of the physician and surgeon in charge, Dr A A McFadyen His hospital is in the centre of the most densely infected area we have been able to find in China and the co operation of Dr McFadyen and others of his mission has enabled us to get a rapid and intimate contact with the surrounding infected villages

As elsewhere, kala azar in China is strikingly and almost exclusively confined to villages Within two or three miles of the city gates we were able to establish a village field station and free treatment centre and consequently to visit freely the infected households

It seemed worth while to begin by means of new methods a review of the insects against which there was suspicion of implication as transmitters of kala azar—the flea and the bed bug At the outset the epidemiological observations made in other countries as well as by ourselves in China made both the flea hypothesis of Basile and the bed bug hypothesis of Patton seem improbable but deserving of re study

In the village of Shih Tzu Shan where kala azar exists endemically *Pulex irritans* was found to infest man and domestic animals In the city (Hsuchowfu) *Xenopsylla cheopis* and *Ceratophyllus* sp probably *fasciatus* were found on rats and *Ctenocephalus felis* on cats All attempts to transmit kala azar by fleas were unsuccessful whether from infected to normal hamsters by adult fleas, or by adult

* Assisted by grants from the China Medical Board of the Rockefeller Foundation

fleas fed with Leishman Donovan bodies (infected spleen juice) during the larval stage

In the bug experiments bed bugs (*Cimex lectularius*) from human habitations and *Cimex pipistrelli* from a common bat were used(4) The method employed with these fleas and bugs as well as all other insects mentioned later except *Phlebotomus* and *Culex* was as follows An unglazed earthen pot about nine inches in diameter with a narrower mouth was used to hold two semi cylindrical cages containing respectively tested positive and negative hamsters insects of the species selected were placed in the pot vaseline smeared around the mouth of the jar and a cover of muslin tied securely in place Such hamsters survived for months sometimes for more than a year During such a period the insects bred It seems probable that both animals were bitten repeatedly by the same insects and that in the case of natural transmitters infection of the negative animal would have taken place Nevertheless with one exception previously unpublished the results were uniformly negative In this instance in a pot containing a pair of striped hamsters the negative animal was shown to have become positive 248 days after the beginning of the experiment We do not claim in the face of negative results in numerous other experiments that *Cimex lectularius* is a transmitter of kala azar but prefer to state our findings thus In one pot in which infected and normal hamsters were present in the known presence of *Cimex lectularius* the negative animal became positive

Before these experiments were completed the striking susceptibility of the Chinese striped hamster (*Cricetulus griseus*) to infection with *Leishmania donovani* had become apparent(1 2 and 3) Its distribution as far as then known was roughly that of the area reported endemic for kala azar in China so that the following hypothesis suggested itself as conforming to the observed epidemiological facts Some rodent perhaps the hamster may be the reservoir of *Leishmania donovani* A seasonal migration due to flood or food scarcity may cause it to enter houses (*cf* plague rats in Java) and there either directly or indirectly through domestic rodents kala azar may be transmitted to man by rodent ecto parasites In order to test this hypothesis the following studies were carried out

1 Tests for the susceptibility to kala azar of house and field rodents in the endemic region(5) Results —The Chinese striped hamster (*Cricetulus griseus*) the giant hamster (*C triton*) and a vole (*Microtus* sp) are highly susceptible to infection with *Leishmania donovani* The Chinese house mouse (*Mus wagneri*) is equally so The black rat (*Rattus rattus*) is moderately susceptible

2 A search for field and house rodents naturally infected with kala azar(6) One striped hamster out of 4 480 was found infected The incidence among these hamsters (0 02 per cent) was only 1/250th of that among the inhabitants (5 per cent) of nearby villages It can, therefore not be maintained that hamsters are the source of human kala azar Since the above series about 10 000 additional striped hamsters have been examined in the course of our work No naturally

infected animals have been found All of the 400 giant hamsters and 134 voles examined were negative None of the 400 black rats and 114 mice trapped in 'clean' houses as well as the 210 black rats and 57 mice from 'kala azar' houses was infected

3 No field rodents were trapped in village houses so that there is no evidence of invasion of human habitations by rodents from the fields

4 Ecto parasites from field rodents were examined for natural infection with *Leishmania* and were also tested as to their ability to transmit kala azar The striped hamster and the giant hamster each has its specific hematopinus louse(7) There are at least three species of mites on striped hamsters Both lice and mites were negative for natural infections with flagellates and all attempts to transmit kala azar from hamster to hamster by the pot method were negative However, we were able to demonstrate in three instances that *Leishmania donovani* is taken up by *Hematopinus* and survives in its body for these lice from infected hamsters, teased up and injected into tested negatives produced infection Obviously these were not transmissions They merely afford evidence that *Hematopinus* is able to ingest the parasites and that the latter survive and are pathogenic under the conditions of the experiment Similar tests with mites were entirely negative No fleas were found on hamsters nor on voles

Our field rodent—ecto parasite hypothesis—therefore received no substantiation whatever when put to the test

Several hundred Culicoides captured in endemic villages were injected intraperitoneally into negative hamsters No infections followed

Stimulated by the work of the Sergents in Algiers on oriental sore(8) we began the study of Chinese sandflies in 1924 The findings of Napier Knowles and Smith(9) published a few months later led us to devote most of 1925 1926 and 1927 to these interesting insects Three species were found (*a*) *Phlebotomus major* var *chinensis* Newstead and two species not previously described for China Specimens sent to Professor Newstead were identified by him as (*b*) *P perturbans* var Patton and (*c*) *P sergenti* var Newstead

A method for breeding these three species was developed(10) and also an artificial method(11) for feeding them with Leishman Donovan bodies which gave a much higher infection rate than those that followed natural feeding either on patients or on infected hamsters Attempts(12) to transmit the disease by means of *P major* var *chinensis* and *P sergenti* var have given only negative results, although 76 of the hamsters were bitten by sandflies subsequently proven to have been infected On the other hand inoculation of infected sandflies intraperitoneally into hamsters produced infection in 23 per cent of the 180 hamsters injected The inoculations were made one to ten days subsequent to the infecting feed

Wild sandflies (443 *P sergenti*) mostly collected from kala azar homes, were injected intraperitoneally into negative hamsters No infections resulted

always proved to have been imported ones from kala azar stricken areas (B India, China) (2) during many centuries there has been immigration of people from India to the Malay Archipelago

Now, on the other hand, the *Phlebotomus* occurs exceedingly rarely in the Malay Archipelago Specimens (I do not remember the species) have been described by de Meyere, but during a 20 years stay in these countries I never have been bitten by a sandfly, nor have I been able to catch a specimen, whereas native people, on being shown a Psychoda have assured me that such an animal could not bite This is only a small bit of epidemiological evidence but if of any value, it is decidedly in favour of the *Phlebotomus* carrier thesis

Major H E Shortt I M S (B India) In the first place, I wish to record my appreciation of the very excellent account given us by Col Knowles of the present position with regard to the problem of kala azar transmission There are some points in his address to which I should like to call attention

(1) Following Napier he lays down certain essential attributes as necessarily possessed by any insect vector of kala azar, viz

(*a*) A long life or the capacity of transmitting its infection to the next generation Now if *P argentipes* be the vector, it possesses neither of these attributes

(*b*) An inability to cross broad rivers the sea or to extend its range above a height of 2 000 feet ' If this is so, it seems to me difficult to explain the existence of kala azar over a range extending from Western Europe to Eastern Asia Moreover, *P argentipes* the probable vector in India, has recently been found in the Himalayas at a height of nearly 6 000 feet

(2) In speaking of the discovery that flagellation of *Leishmania* occurs in the sandfly *P major* var *chinensis* in China Col Knowles attributed the discovery to Patton and Hindle, whereas they were merely corroborating the previous work of Young and his collaborators

(3) On one point I have to differ definitely from Col Knowles He makes the statement that in India the Chinese hamster has not been found more susceptible than the white mouse My experience in Assam admittedly with small numbers has been that, of all the laboratory animals experimented with, the Chinese hamster is unquestionably the most susceptible to *L donovani*

(4) I think the most important part of Col Knowles' paper deals with the subject of resistance in man and animals exposed to infection He stresses the important role which may be played by diminished individual resistance due to economic stress other diseases and endocrine disturbance This is a question upon which at present we really have no knowledge A feature of kala azar in Assam is that of ' family infection ' It is a common place for every member of a family to become infected, not necessarily all at the same time This would imply the operation of the factors mentioned on all members of the family, and the same would apply on a larger scale to cases where every house in a village becomes affected, a not infrequent occurrence in Assam

Dr S L Sarkar (Bengal) Stated that there are epidemic years for kala azar as in malaria There was a kala azar survey made of certain villages of the Noakhali District by Dr S N Sur, Assistant Director of Public Health, about six years ago The

speaker found that this epidemic increased markedly about the year 1925 and then declined Of course, systematic injections for the treatment of kala azar organized in the District may have gradually had some effect

He also noticed, like Dr Young, that kala azar cases might be small in number in a town, but very numerous in the villages situated very close by In the villages, the speaker noticed foci of kala azar cases in definite parts of the district These cases were confined in houses grouped and clustered together There was often a patch of jungle, or the ruins of a building or hut in the neighbourhood But while the people of the group of huts were infected, the rest of the villagers were free In a district the speaker also noticed that a village situated on an alluvial soil, might be almost completely infected, the rest of the villages in the neighbourhood remaining comparatively free

He found many kala azar cases coming for treatment heavily infected with hookworm It was, in the speaker's opinion a question worth investigating whether kala azar cases could be successfully infected from persons infected with hookworm

Dr L Everard Napier (Bengal) I should like to make a few remarks on points that have been raised Major Shortt referred to what Col Knowles has chosen to call my 21 cardinal points, he said that the sandfly did not fall under either the category of an insect 'with a long life or capability of transmitting the infection to the next generation,' or of one 'that was incapable of crossing broad rivers or the sea or extending its range above a height of 2 000 feet' I am inclined to agree with Major Shortt It must be remembered that these points were deduced from a study of the epidemiology of the disease, and not from a study of the life history of the sandfly It is extremely unlikely that any insect would fulfil these 21 requirements If one can be found that will fill nearly all of them, as does the sandfly then I think that this insect falls under very strong suspicion as the transmitter of kala azar My answer to Major Shortt is 'if you know a better 'ole, go to it' or rather if he knows of a better insect, please tell us about it'

I should like to defend Col Christophers against Major Shortt's accusation that he (Col Christophers) observed the condition dermal leishmaniasis in kala azar patients in Assam many years ago without realizing what he was looking at He reported the finding of papules on kala azar patients in which leishmania were found Now amongst the various types of dermal leishmaniasis described by Col Acton and myself papular eruptions were not included furthermore on only one occasion has the condition been observed in patients suffering from a visceral leishmania infection at the time, although they have almost certainly had a visceral infection at some time or other

Lieut Col J H D Megaw I M S (Bengal) Congratulated Dr Young on his brilliant work and on the remarkably ingenious apparatus which he had devised for the artificial feeding of sandflies He considered the time has come to take stock of the existing position with regard to the transmission problem Some workers were inclined to adopt a purely pessimistic and negative attitude towards the sandfly hypothesis they had started hot on the scent of the sandfly, they encountered checks and then some of them were inclined to give up the chase

He submitted that such checks are not surprising the observed distribution of kala azar suggests that some serious obstacle exists to the communication of the disease from man to man, otherwise it would be far more prevalent than it is at the present

time There are certain places in which the disease has excessive prevalence, there are other places in which it is rare or unknown He suggested that work should concentrate on finding out what conditions exist in the places where the disease is prevalent which are absent in those places where it is rare If we can discover the obstacle which prevents the sandfly from being effective we may succeed in transmitting the disease to man under experimental conditions We should not commit ourselves to the view that there is no other possible means of transmission of the disease The difficulty at the moment is that nobody has suggested any other mode of transmission which has the smallest evidence to support it The position then is that we have the sandfly hypothesis which fits in with the known facts but has not been proved experimentally in the absence of any other promising suggestion we have to persevere in the efforts to discover the obstacle which exists in preventing the effective experimental transmission by the sandfly

Lieut Col W C Ross I M S (Bihar and Orissa) I wish to put forward a plea for the greater consideration of the epidemiological evidence with regard to the transmission of kala azar I do not wish to criticize the work of the research workers except on one point which is that it appears to have been assumed too readily and too soon that transmission must be by a biting insect and that the sandfly *argentipes* is the most probable vector I wish rather to draw attention to the different conditions and epidemiological factors which exist with regard to kala azar In Assam kala azar is primarily an epidemic disease which has not yet settled down into being or becoming really endemic In short it is a new disease in Assam whereas in Bengal and in Bihar it is an endemic disease My own experience of it in Bihar is that it is an endemic disease so typically that it must have been there for a long time It has none of the characteristics of a *recent infection* There is *therefore much valuable epidemiological evidence to be* obtained in such an area The disease is very prevalent and is responsible for a large number of the deaths returned as deaths from fever The heading 'death from fevers' is one which is very misleading Malaria in Bihar is the least important factor under this head which is much more filled up by deaths from pneumonia, enteric fever and kala azar and there is little doubt but that kala azar is an important cause of death in Bihar

I would further suggest that economic and climatical influences play *a very* important part with regard to the prevalence and the mortality from kala azar just as they do in other diseases and that these require very careful examination and *consideration*

I had a very *detailed kala azar survey made of a large area in Patna city with a* population of about 35 000 and from that I obtained a great deal of valuable information which I am at present writing up for publication I quote some of the more outstanding points which are first, that kala azar is a very rare disease below the age of two years and is not common under five The curve of prevalence in age incidence then rises rapidly to 15 falls slightly to 20 and then falls If we, for the moment, ignore the *first point with regard to young children* the curve is exactly the same as the enteric curve in Europe and in America which is a point of great interest and possible if not probable importance The fact that the disease is rare amongst young children is most important Malaria which is known to be transmitted

by a biting insect occurs at very early ages, whereas kala azar does not There is, therefore, a strong probability that the disease is not transmitted by a biting insect at all It is true that the incubation period may be a long one but it is equally true, and of greater importance that the incubation period is usually comparatively short in the great majority of cases Further I have found and I know that several other observers have also found that kala azar is much more prevalent amongst Mohammedans than amongst Hindus In Bihar the ratio is at least 2 5 to 1 Hindus and Mohammedans are equally liable to be bitten by biting insects and they are equally liable to infection from water supplies but they have very different customs with regard to their food Hindus cook each meal separately and eat it at the time and they discard any food that remains Mohammedans cook when it suits and keep cooked food all day or even for one or two days under conditions which expose it to contamination That is in my opinion the most important characteristic difference in their habits of living

There is a further observation of great importance with regard to housing In the area surveyed in Patna there were mud huts with grass roofs mud houses with tiled roofs and brick built houses The social and economic condition of the inhabitants varied with the style of house and one would have expected that the poor and ill fed people living in the mud huts should have been the greatest sufferers Such however is not the case but rather it is the exact opposite The brick built pucca houses show the greatest prevalence of kala azar and the poorest mud huts the least That fact appears to indicate that kala azar is not a disease associated with bad housing

I have quoted three definite observations in an epidemiological survey on a large scale which are very difficult to reconcile with the theory that kala azar is transmitted by a biting insect I have more evidence but I have not now time to discuss it I will therefore conclude by suggesting that any theory which is really correct and true must and will tally with all the observed facts and that it is desirable to examine all the facts with a view to finding out that which is true and not in the hope of being able to reconcile them with the theory

Dr Lal Behari Ganguly (Bengal) Asked if Dr Young had used cultures of the parasite or leishmania forms in his experiments

Dr U N Brahmachari (Bengal) A few years ago the bed bug was supposed to be the carrier of the disease and opinion was expressed by such a high authority as Patton that there was very little doubt about this theory The bed bug theory was now exploded

Col Megaw has asked whether an insect other than the sandfly would fit in with all the known factors regarding the incidence of the disease We may not know such an insect today but it may be discovered in the future if a blood sucking insect is really the carrier of the parasite of kala azar The various factors regarding the incidence of the disease described by Dr Napier to which Col Knowles has just now referred appear to me to raise an extremely complex and perplexing problem which may be impossible to solve Many of these factors may have little to do with the incidence of the disease For instance the statement that the disease does not exist 2,000 feet above the sea level will soon have to be revised when it has been observed that the disease occurs endemically in such a place as Sanawar in the Simla Hills, which is 5,700 feet

above the sea level and many of the other conditions laid down by *him may not hold* good in the case of Sanawar The disease may sometimes occur in the interior of Calcutta where some of the supposed factors are absent

Reference has been made by Col Knowles to dermal leishmanoid a disease originally observed by me and to transient infection with *Leishmania donovani* When dermal leishmanoid was discovered it struck me that the skin was probably a channel of infection *from man to man* When subsequently such lesions in the skin were discovered by Acton and Gupta in cases that gave no history of kala azar I then threw out a suggestion *that there* were probably cases in which an immunity of the internal organs had taken place against their infection with *Leishmania donovani* The question now arises as to what is the mechanism of this immunity, which stands as a barrier between a *very mild* skin infection as shown by small patches of a pigmentation and a most virulent disease i e kala azar or internal leishmaniasis in which the death rate was held to be more than 90 per cent in the pre antimony days

It is hard to have to yield to the view that transient infection of the internal organs with *Leishmania donovani* may take place in man without subsequent development of kala azar If that were so then one would have met with a large percentage of spontaneous cures in cases met with in their earliest stages but it is not so

One fact that I would refer to is that not infrequently kala azar and typhoid fever may manifest themselves in an individual at one and the same time and it may be possible that infection with both the diseases may have taken place through the gastro intestinal tract If that be the case then the time has probably arrived when the whole situation may have to be reviewed and the attention of research workers may once more be drawn to the gastro intestinal tract as a possible channel of infection especially in view of the fact that the recent observations of Perry Meleney and others have shown that there may be an intense infection of the gastro intestinal tract by means of *Leishmania donovani* in post mortem findings It may be argued that a patient suffering from what may be termed latent kala azar for an indefinite period may manifest symptoms of the disease during an attack or after an attack of typhoid fever These are problems that have still to be solved To my mind much work will have to be done before the proper mode of infection of the disease is settled once for all and the factor of resistance worked out more fully

Dr P I Gharpure (Bombay) Dr R Rows work has shown the following points as seen by me in the Petit Laboratory Bombay —

Resistance of Mice (Japanese variety) —

A *Leishmania tropica*

(1) Mice infected by the intraperitoneal route with material from soils rarely show successful infections

(2) In the few successful infections the parasite count was so poor that film examination failed to show the presence of the parasites but a culture was positive and passage produced a severe general infection On successive passages the virus ultimately dies out Very successful inoculation takes place by using old cultures

(3) Continuous infiltration has failed to produce lesions

B *Leishmania donovani*

(1) Mice are very susceptible (as can be judged from inoculations from human isolated cases imported into Bombay) The virulence goes on continuously increasing until the life of the mouse is shortened to three months

(2) Old cultures give both cutaneous and general infections in monkeys and general infections in mice

C *Transmission*

Oral —Splenic material given as a feed fails to produce either changes in organs or parasitic infection Larvæ fed on spleens of infected mice given to mice per os produce enlargement of spleen and liver but the parasites are not found in either spleen liver or bone marrow Subsequent passages of spleen pulp by intraperitoneal infection fail to show either changes in the organs or presence of parasites

In laboratory transmission by intraperitoneal injections an injection of spleen pulp gives a more severe infection than an emulsion of spleen and liver both together

Bt Col S R Christophers I M S (B India) Said that he was very surprised to hear from Major Shortt that he (Col Christophers) had discovered dermal leishmaniasis His hopes however had soon been dashed by Dr Napier He had practically no experience of the condition himself as he had not had opportunity of seeing such cases as were now so well known in Calcutta At the time Major Shortt referred to he was mainly concerned with the distribution of the parasite in different tissues of the body and that they were to be found at all in skin lesions in kala azar he was afraid was all that the observations made at that time meant

The remarks of several speakers had indicated a doubt as to the sandfly being the carrier after all but so much pointed to this insect that to him this seemed a wrong position to take up That a difficulty had arisen in getting actual transmission had probably to be looked for in still unknown conditions determining the onset of infection Major Shortt had shown the very great probability that flagellates were actually passed into the wound by the biting sandfly As we know these flagellates were actually leishmania the hitch was not in transmission so much as in want of development of infection

Some speakers had spoken of a return to still unknown methods of transmission such as the contaminative theory and to an as yet undiscovered insect host Regarding the first he had himself undertaken as directing the Kala azar Commission s work, before the discovery by Knowles Napier and Smith extended observations as to the viability of the parasite etc in external circumstances and their work was in opposition to any such view as a contaminative method of transmission In Madras city the crowded parts of the town were so densely inhabited that no wild or unknown human biting insect transmitter seemed at all probable Work so far on known forms of human biting insects the bug Conorhinus the mosquito etc had failed but with the sandfly the case of infection was remarkable There was in addition the fact of apparent danger in the forward development of the parasite in the alimentary canal of the sandfly and other facts supporting the probable role of this insect, such as the part played seemingly by the sandfly in the transmission of oriental sore The line of investigation at present, therefore, would appear to be along the study of the reasons

for failure of infection to develop after the bite of the infected sandfly rather than in a return to the original state of complete doubt as to the channel of infection

Prof J W W Stephens (Gt Britain) Said that some time ago he had put forward the suggestion that the disease might not be transmitted from one human being to another

Dr C W Young (N China) in reply to Major Shortt There was every chance of contamination of the negative hamster with the body fluids (urine and fæces) in our Cimex experiment There was also the possibility of direct infection into slight wounds I may add that we have infected hamsters by the intraperitoneal injection of the urine from infected hamsters

I should like to call your attention to the fact that all of the sandflies naturally infected with *Leishmania tropica* caught by Adler and the Sergents, were from houses in which there were no cases of oriental sore Moreover Sergent's infected sandflies were caught at a time when there were no cases of oriental sore present in Biskra

In reply to Dr Gharpure I have here two animals one white mouse and one hamster infected with *Leishmania tropica* by injection of cultures of the flagellates into the base of the tail and scrotum The cultures were obtained from Drs Visolle and Anderson in Tunis The strain has been under artificial cultivation for a number of years We have tried infection by the gastro intestinal route, by feeding negative hamsters on carcasses of infected (kala azar) hamsters All attempts were unsuccessful

In reply to Dr Ganguly The series of subcutaneous inoculations in hamsters was with saline suspensions of infected spleens and therefore was with the *Leishmania donovani* form

Major H E Shortt I M S (B India) in his reply said that the discussion had not centred round his paper

Lieut Col R Knowles I M S (Bengal), replied There are several points which I have to answer,

First I emphatically agree with Dr Young that intraperitoneal inoculation does not correspond to any natural channel of infection *I should like to stress this point* because I do not think that intraperitoneal inoculation experiments are going to help us to solve the problem

Secondly with regard to the age incidence of Mediterranean kala azar it is a fact that this disease almost exclusively affects children under two years of age and this fact may have a most important bearing on the epidemiology of the disease I wish the Mediterranean workers would find out the reason for this incidence, and explain it [illegible] the problem With regard to the hamster, [illegible] not hibernate at [illegible] its bodily activities are lowered and this may give the parasites a chance for [illegible] development

Finally *I should like to say that I have never seen more typical sandfly country than the* infected Chinese villages shown in Dr Young s most beautiful lantern slides You have ideal conditions for the sandfly there fæcally polluted earth, vegetation shade and moisture

With regard to the longevity of *P argentipes*, Major Shortt himself has sh its longevity is greatly extended during the monsoon months and that it will long enough to transmit the infection With regard to his question about co of the disease overseas I believe I am right in saying that an outbreak of always begins by the importation of an infected human being into the area that Col McCombie Young with his many years of experience of the disease will confirm that statement I do not believe in the animal reservoir theory I have tried to investigate it It is the *human* host that constitutes the sour infection, and is the important factor With regard to the question of lowered re I do not claim that this consists only in endocrine deficiency it may be brou by intercurrent disease semi starvation or any other debilitating factor

With regard to Dr Napier's remarks on dermal leishmanoid this dise common in Calcutta that we are accustomed to see 10 or 12 cases a month a in only one case have we seen the co existence of kala azar with it This was female patient seen by Dr Das Gupta She first developed dermal leishma lesions being chiefly on the nose Six months later she developed fever and *L* was cultivated from her peripheral blood As a rule dermal leishmanoid and cannot co-exist at the same time in the same person

With regard to Col Megaw's remarks I am so convinced that P argentip vector that I am going to make a definite suggestion I suggest that in 1928 azar Commission take two adjacent areas each about equally affected with k that during the monsoon months of 1928 they apply the most vigorous anti measures in one area, leaving the other as an untreated control They will the in 1929 *to study the results of these measures*

Prof Stephens does not believe that kala azar is transmitted at all fro man I presume that he refers to the view at one time entertained by Laveran a that leishmaniasis like diseases can be produced by injection of insect herpet into vertebrate hosts But the recent and exhaustive studies by Hoare, Shortt above all and Drbohlav appear to entirely negative that view All the ments had negative results and the view that *L donovani* is an insect herpe normally transmitted from insect to insect and only occasionally inoculated appears to have no foundation in fact The natural herpetomonads of insect to have no relationship to the leishmania parasites in herpetomonas infec infection is transmitted from man to man by the posterior route the infecti being passed in the fæces, in the leishmania infections development procee forward direction infection being introduced by the bite It is the human fa counts in an epidemic as the source of the virus

Col Ross and several other speakers still appear to favour the contaminatio a theory which I had hoped was entirely dead (*Col Ross*—No that is w do not entertain any theory I only want more epidemiological study of the (*Col Knowles*)—I am sorry Anyhow there seemed to be considerable among the audience for the contamination theory and I am sorry to note it convinced that it is wrong If you turn to consider the transmission of kala a

investigating that possibility and found nothing Col Christophers did the sa so have others *Leishmania donovani* is extremely susceptible to sepsis, and woul at once killed out in the septic environment of the fæces If there is any special se resistant form of the parasite will the supporters of the contamination theory ki find that form and demonstrate it to us? It is true that Shortt and others have sh that the parasite can occasionally be recovered in culture from the urine, but incredible that it could survive in the septic environment of the soil

On the other hand the parasite can always, or almost always—in 95 per cen cases at least—be cultivated from the peripheral blood, and that means transmis by a biting insect

Turning to the other end of the transmission cycle we must answer the questio how the parasite gets into man It must be either by ingestion or injection Sev workers have suggested that infection is acquired by the oral route, and I had *monkey who apparently contracted the infection in that manner* But in giving feed of spleen juice we had to use a mouth gag and the monkey's gums were rat severely abraded, and I am now inclined to think that it was by this channel that infection was acquired The Kala azar Commission have carried out a very large se of feeding experiments and the results were completely negative They seem to h definitely ruled out infection by ingestion There only remains infection by injecti For these reasons I believe the contamination theory to be ruled out I was very m interested in Col Ross remarks on the correspondence between the age incidence cu for enteric fever and that for kala azar I consider enteric fever to be one of the m important predisposing causes of kala azar, and therefore this correspondence is v interesting

Col Ross asks me whether *P argentipes* can discriminate between a Mohammec and a Hindu? Well, funnily enough I think that it does I hope it has not beco inoculated with the communal virus! The fact is that practically every Hindu hou hold keeps a cow whereas the Mohammedans do not Now Napier and Lloyd ha shown by serological tests that *P argentipes* will feed every time by preference the cow rather than on man In northern Calcutta the population are chiefly Hindu and they all keep cows Now *P argentipes* requires fæcally polluted soil in which lay its eggs In northern Calcutta this insect lays its eggs in the soil of cowsheds an when the mature insects emerge they feed on the cows Hence although kala azar repeatedly imported into northern Calcutta the disease does not spread

In the endemic area in Ward 14 however, the population are chiefly Anglo India and Mohammedans, and they do not keep cows but keep ducks and fowls He *P argentipes* breeds in soil polluted with the droppings of ducks and fowls Whe the adult insects mature, there are no cattle for them to feed upon and hence the take to feeding on man For this reason kala azar spreads in this area I trust th I have now explained how it is that *P argentipes* comes to prefer the Mohammeda to the Hindu (*Col Ross*—In the rural areas in Bihar the Mohammedans keep ju as many cows as the Hindus do) (*Col Knowles*)—I am glad that Dr Brahmacha has mentioned the Sinawar cases in a hill station, because Major Sinton has informe me that they have now found *P argentipes* there Here we have a most importar

with it you find an isolated crop of kala azar cases, presumably following after the importation of an infected person When Dr Napier wrote his epidemiological papers we had not then had the information about the occurrence of these sporadic cases at high altitudes

I was very much interested to hear Dr Gharpure s account of his experiments with *L tropica* With regard to his suggestion of invasion by ingestion, however I think again that the work of the Commission rules that out

I was very glad to hear Col Christophers' remarks This is certainly not the moment to abandon work on so promising a line of work as that on *P argentipes* In our experience the midgut of *P argentipes* forms a far and away more favourable culture medium for *L donovani* than any culture medium—NNN or other—that I know of I have never been able to find in Patton s papers the proportion of the bed bugs which he fed which became positive but I understand from his colleagues who worked with him that it was a very small one On the other hand with *P argentipes* it is a very high one, you get 30 to 40 per cent of flies positive after feeding

In conclusion, it is evident that there is some hitch in the final proof of sandfly transmission Well, that hitch is not in the sandfly part of the cycle for Major Shortt has worked that out very fully Indeed he has gone further than I knew, for one of his lantern slides shows the actual injection of flagellates into the wound (*Major Shortt*—No That is wrong Only infection of the buccal cavity) (*Col Knowles*)— I am sorry Well, anyhow, the solid plug of flagellates in the pharynx the infection of the buccal cavity and the inevitable injection of the flagellates by the fly when feeding So that the fly cycle is now completely known

The hitch therefore, is in the human part of the cycle It lies, I believe, in the factor of human resistance to infection, and I believe that our next step must be to investigate and study that factor and find out how to break it down

investigating that possibility, and found nothing Col Christophers did the *same*, so have others *Leishmania donovani* is extremely susceptible to sepsis, and would be at once killed out in the septic environment of the fæces If there is any special sepsis-resistant form of the parasite, will the supporters of the contamination theory kindly find that form and demonstrate it to us ? It is true that Shortt and others have shown that the parasite can occasionally be recovered in culture from the urine, but it is incredible that it could survive in the septic environment of the soil

On the other hand the parasite can always, or almost always—in 95 per cent of cases at least—be cultivated from the peripheral blood, and that means transmission by a biting insect

Turning to the other end of the transmission cycle, we must answer the question of how the parasite gets into man It must be either by ingestion or injection Several workers have suggested that infection is acquired by the oral route, and I had one monkey who apparently contracted the infection in that manner But in giving the feed of spleen juice we had to use a mouth gag, and the monkey's gums were rather severely abraded, and I am now inclined to think that it was by this channel that the infection was acquired The Kala azar Commission have carried out a very large series of feeding experiments, and the results were completely negative They seem to have definitely ruled out infection by ingestion There only remains infection by injection For these reasons, I believe the contamination theory to be ruled out I was very much interested in Col Ross' remarks on the correspondence between the age incidence curve for enteric fever and that for kala azar I consider enteric fever to be one of the most important predisposing causes of kala azar, and therefore this correspondence is very interesting

Col Ross asks me whether *P argentipes* can discriminate between a Mohammedan and a Hindu ? Well, funnily enough, I think that it does I hope it has not become inoculated with the communal virus ! The fact is that practically every Hindu household keeps a cow, whereas the Mohammedans do not Now Napier and Lloyd have shown by serological tests that *P argentipes* will feed every time by preference on the cow rather than on man In northern Calcutta the population are chiefly Hindus, and they all keep cows Now *P argentipes* requires fæcally polluted soil in which to lay its eggs In northern Calcutta this insect lays its eggs in the soil of cowsheds, and when the mature insects emerge they feed on the cows Hence, although kala azar is repeatedly imported into northern Calcutta, the disease does not spread

In the endemic area in Ward 14, however, the population are chiefly Anglo Indians and Mohammedans, and they do not keep cows, but keep ducks *and fowls Here P argentipes* breeds in soil polluted with the droppings of ducks and fowls When *the adult insects mature, there are no cattle for them to feed upon* and hence they take to feeding on man For this reason kala azar spreads in this area *I trust that* I have now explained how it is that *P argentipes* comes to prefer the Mohammedan to the Hindu (*Col Ross*—In the rural areas in Bihar the Mohammedans keep just as many cows as the Hindus do) (*Col Knowles*)—I am glad that Dr Brahmachari has mentioned the Sanawar cases in a hill station because Major Sinton has informed me that they have now found *P argentipes* there Here we have a most important and interesting finding, *you find a patch of argentipes* infection at high altitude, and

with it you find an isolated crop of kala azar cases, presumably following after the importation of an infected person When Dr Napier wrote his epidemiological papers we had not then had the information about the occurrence of these sporadic cases at high altitudes

I was very much interested to hear Dr Gharpure's account of his experiments with *L tropica* With regard to his suggestion of invasion by ingestion however I think again that the work of the Commission rules that out

I was very glad to hear Col Christophers remarks This is certainly not the moment to abandon work on so promising a line of work as that on *P argentipes* In our experience the midgut of *P argentipes* forms a far and away more favourable culture medium for *L donovani* than any culture medium—NNN or other—that I know of I have never been able to find in Patton's papers the proportion of the bed bugs which he fed which became positive, but I understand from his colleagues who worked with him that it was a very small one On the other hand, with *P argentipes* it is a very high one, you get 30 to 40 per cent of flies positive after feeding

In conclusion it is evident that there is some hitch in the final proof of sandfly transmission Well, that hitch is not in the sandfly part of the cycle for Major Shortt has worked that out very fully Indeed, he has gone further than I knew for one of his lantern slides shows the actual injection of flagellates into the wound (*Major Shortt*—No That is wrong Only infection of the buccal cavity) (*Col Knowles*)—I am sorry Well, anyhow, the solid plug of flagellates in the pharynx, the infection of the buccal cavity and the inevitable injection of the flagellates by the fly when feeding So that the fly cycle is now completely known

The hitch, therefore, is in the human part of the cycle It lies, I believe, in the factor of human resistance to infection and I believe that our next step must be to investigate and study that factor and find out how to break it down

THE EXPERIMENTAL TRANSMISSION OF ORIENTAL SORE (CAUSING GENERALIZED INFECTION) IN LABORATORY ANIMALS

BY

B M DAS GUPTA

Assistant Professor of Protozoology Calcutta School of Tropical Medicine

WHILST working on the serological tests for differentiating species of *Leishmania* obtained from cases of dermal leishmanoid (Brahmachari 1922) from *L donovani* and *L tropica* according to the technique advocated by Noguchi (1924) very rich growths of the herpetomonad forms of all three organisms were obtained in the water of condensation of NNN medium

The water of condensation from eleven such rich NNN cultures of *L tropica* obtained from a case of oriental sore was pooled and injected into a young guinea pig and two (English) white mice The guinea pig received 3 5 c cs intraperitoneally white mouse No 1 received 0 5 c c subcutaneously and No 2 received 0 5 c c intracutaneously distributed in three points of injection into the shaved skin of the abdomen The guinea pig developed a nodule 20 days later at the site of injection and this persisted until the animal was killed Scrapings of the nodule were examined from time to time but only showed scanty streptothrix and small oval bodies possibly yeasts or bottle bacilli Cultures from the nodule on NNN showed only contamination present

The guinea pig was killed on the 92nd day after injection Smears from its spleen liver kidney and bone marrow showed numerous leishmania present in all these organs There was no appreciable enlargement of its spleen however

White mouse No 1 inoculated subcutaneously never showed any local lesion It was killed on the 92nd day Very scanty leishmania were found in the smears from its spleen The spleen was considerably enlarged

White mouse No 2 inoculated intradermally showed diffuse induration all over the abdomen a few days after the injection but this disappeared at the end of a week It was also killed on the 92nd day but no leishmania could be detected in any of the smears from its different viscera though its spleen was much enlarged Cultures on NNN medium from its liver, however gave a scanty growth of flagellates on the 11th day so that this animal also had contracted a visceral infection

Cultures on NNN from the viscera of the guinea pig and of white mouse No 1 gave a good growth of flagellates on the sixth day

These experiments confirm the susceptibility of small laboratory animals to infection with *Leishmania tropica* their susceptibility to this species being much greater than their susceptibility to infection with *Leishmania donovani* Whilst *working at the Pasteur Institute at Shillong some eight years ago I tried to infect* a guinea pig with *L donovani* the animal being given a massive dose of spleen emulsion full of Leishman Donovan bodies the viability of which was proved by cultures and by successful inoculation into monkeys This attempt completely failed whilst a number of white mice injected subcutaneously with cultures of *L donovani* also escaped infection

These results appear to differ from those of Laveran (1915) in whose experiments cutaneous lesions were almost always produced by injections of *L tropica* at first followed by a generalized infection of the internal viscera later They agree however with those of Row (1924) with *L tropica* where systemic infections were produced in all his mice without any skin lesions resulting after repeated intraperitoneal injections

REFERENCES

BRAHMACHARI U N (19[illegible]) A new form of cutaneous leishmaniasis—dermal leishmanoid *Ind Med Gaz* Vol LVII p 12[illegible]

LAVERAN A (1915) Nouvelle contribution à l'étude des infections expérimentales de la souris par *Leishmania tropica* *Bull Soc Path Exot* Vol VIII p 363

NOGUCHI H (19 4) Action of certain biological chemical and physical agents upon cultures of Leishmania some observations on plant and insect herpetomonads *Proc Internat Conf Health Problems in Trop America* Boston Mass p 455

ROW R (1924) On some pathological observations in experimental leishmaniasis in mice with special reference to generalized infections set up by *Leishmania tropica* *Ind Jour Med Res* Vol XII p 4[illegible]

THE ACTION OF THE PENTAVALENT COMPOUNDS ON THE *LEISHMANIA DONOVANI* PARASITES

BY

LIEUT COL H W ACTON I M S

Professor of Bacteriology and Pathology

AND

LIEUT COL R N CHOPRA M A, M D (Cantab) I M S

Professor of Pharmacology,

Calcutta School of Tropical Medicine and Hygiene

DURING our researches on the mode of action of specific drugs one of us (R N C) noted the very marked effect that certain pentavalent compounds of antimony notably urea stibamine and amino stiburea had on the volume and contraction of the spleen when compared with compounds like antimony tartrate We considered that this effect should in some way account for the better therapeutic results that have been obtained in the treatment of kala azar by these new pentavalent compounds of antimony We decided to investigate how these compounds acted on the leishmania parasites and brought about a cure of the disease We first noticed that they had little or no direct action on protozoa like the *Paramœcium caudatum* as they could live in concentrations like 1 500 that could never possibly be attained in the circulating blood stream, whilst flagellate cultures of the Leishman Donovan parasites could survive in a 1 200 solution of urea stibamine for a considerable time Napier has noticed that some of his cases of kala azar after a course of ten injections with these newer pentavalent compounds were still positive on spleen puncture or on culture of the splenic pulp Such cases usually went on to a permanent cure without further treatment being necessary The cure was therefore not due to the direct action of the drug but that it stimulated some tissue in the body which continued exerting its action and brought about a cure without any further treatment The so called immunity that occurs after a course of Bayer's 205 against trypanosome infections appears to be somewhat analogous to the effects produced by these pentavalent compounds of antimony With Bayer's 205 it is considered that the drug stimulates the reticular endothelial tissues to form some trypanocidal substance in the serum

Before we attempt to discuss the method of action of these compounds of antimony, we will first detail the clinical and experimental data

Clinical

It was at first thought that kala azar was a disease which was very fatal in its effect, and that only about 20 per cent of the untreated cases ever recovered In the pre-antimony days, the main lines of treatment consisted in stimulating the leucocytes by various injections Thus Muir advocated the use of turpentine injections and one of us in January 1914 remembers Sir Leonard Rogers praising the use of a sensitized streptococcal vaccine so it seems that substances that stimulate the bone marrow and produces leucocytosis indirectly help to bring about a cure Napier's work on kala azar has shown that some of the cases that he has diagnosed by blood culture as positive and not been treated have gone on to complete recovery so that there must be some natural defence mechanism present in the bone marrow and other situations that brings about a cure in kala azar

The next point was observed by Napier from his clinical experience of a study of a group of villages at Koarapukar during the last three years The population of these villages consisted of about 1 972 individuals and only about 3 to 18 per cent of the villagers yearly showed clinical signs of kala azar indicating that in spite of being subjected to infection sometimes only a small proportion of the cases actually developed kala azar The following table shows the results of investigations in these seven villages for 1927 —

Table I

Name of village	Number of houses	Population	Kala azar cases in 1926	Percentage infected
Koarapukar	138	565	17	3
Lodabhet	47	358	14	3 9
Kazichawk	28	16	18	10
Ramchandrapur	43	256	19	7 2
Pamnagar	39	206	12	6
Sajuabaria	30	201	6	3
Thakuran chawk	4[illegible]	219	11	5

There is reason to believe that many of these slight and transient infections occur in nature and never develop typical signs such as we recognize as the disease kala azar namely fever enlargement of the spleen anæmia and wasting There is also evidence that certain debilitating diseases like influenza typhoid fever etc, precipitate these transient or ambulatory cases of kala azar into the acute type of the disease This has been previously noted by Napier Knowles and McCombie Young in connection with this disease From clinical evidence therefore we see that the *Leishmania donovani* parasites may infect an individual without necessarily causing the disease a condition we may term transient leishmaniasis' and the parasites only become active when the defence mechanism of the body is *lowered by disease etc* When we come to consider this defence mechanism we will see that it is partly located in the bone marrow and partly in other tissues of the body One of us (H W A) for the last six years has been studying the various skin diseases that occur in the tropics and during the course of this experience he has observed at least 1 000 cases of leucoderma and not one of these cases has ever showed any signs of kala azar On the other hand both of us have seen a large number of cases of kala azar and have never once seen a case amongst them suffering from leucoderma other than that due to direct trauma depigmented scars The word kala azar means *black fever* and many of the cases are associated with a slight excess of pigmentation of the skin During our researches on leucoderma and chloasma Dr J P Bose has examined these cases with regard *to their adrenal function by the insulin and adrenalin test He found that in both* of these conditions the blood sugar is low i e about 0 085 somewhat similar to the European figures but after an injection of adrenalin in the leucoderma cases *there was a considerable rise in the blood sugar but in the chloasma and kala azar* cases the blood sugar level was only slightly altered suggesting that the adrenal content of the blood in the leucodermic cases was higher as a small dose of adrenalin causes a marked dilatation of the portal capillaries which set free the sugar stored in the liver On testing these cases with insulin we found that in the leucoderma cases the blood sugar was only slightly diminished suggesting that the adrenal defence mechanism prevented the development of hypo glycæmia In the chloasma and kala azar cases there was a considerable fall in the blood sugar and many of the cases exhibited symptoms of hypoglycæmia These tests tend to suggest that the leucoderma cases have hyperadrenia whilst the chloasma cases like Addison s disease are due to hypo adrenia In Bengal we get a curious condition which Acton and Napier (1927) have described as post *kala azar dermal leishmaniasis* In these cases leucodermic spots first develop on the chin inner side of the arm and inner side of the thigh followed by granulomatous nodules containing the parasites and after many years plaques of granulomatous tissue develop about the axilla and flexures of the elbow Half of these cases give no history of fever but an equal proportion give a definite history of fever which was *possibly kala* azar and out of the latter cases half i e 25 per cent have undergone treatment with antimony In this condition we have the *Leishmania donovani* parasites

growing for years under the epidermis of the skin and occasionally in these cases one can cultivate parasites from the peripheral blood We have now seen about 100 of these cases and none of them have ever shown any evidence of visceral infection so that the tissue of the spleen bone marrow etc must be resistant to these parasites and the only place that these can grow is in the skin Clinical evidence therefore points to the fact that for infection to occur (1) leucopenia is necessary, (2) that a condition of hypo adrenia is also favourable and (3) the reticular endothelial tissue of the spleen liver and bone marrow is more favourable for the growth of the parasite than the skin

EXPERIMENTAL

The first thing for us to test was the action of these compounds on protozoa and on the *Leishmania donovani* flagellates (*a*) on *paramœcium* we found when using dextro rotatory tartar emetic they lived in a 1 200 dilution for 10 minutes or longer The lævo salt killed them in a 1 500 dilution showing that the lævo salt was twice as powerful as the dextro tartar emetic In weak concentrations such as 1 1000 the paramœcia were able to live for several hours urea stibamine had no effect whatever in a dilution of 1 200 and in 1 100 they were paralysed after 15 minutes but were not dead and this effect was probably produced by the concentration of the drug interfering with osmosis (*b*) on the *leishmania flagellates* With one part of the culture and four parts of a 4 per cent tartar emetic solution, all movements ceased in seven minutes Using a 4 per cent solution of stiburea one part of a culture and four parts of stiburea movement ceased in 48 minutes, whilst with one part of the culture and six of stiburea they were quite active after 30 minutes showing that the antimony did not exert a direct action on the parasites

(1) *Action on the liver and spleen* During the course of our investigation one of us (R N C) found that the action of these compounds particularly the penta valent compounds caused a marked increase in the volume of the spleen with intense rhythmic contractions The same effect but to a lesser degree is also observed in the volume of the liver This dilatation occurred independently of any fall in the general blood pressure showing that the action was directly on the vessels and involuntary muscular cells of these two organs Compounds like stiburea and amino stiburea give rise to a greater increase in the volume of these organs than the other compounds tested by us The next point to determine was whether this effect was due to the antimony acting on the liver or through the adrenals, as the action is very similar to that which occurs after the injection of adrenalin To test this point we occluded the adrenal veins by clamps and then injected these antimony compounds the result was that there was practically no increase in the volume of the liver or spleen The experiment was repeated by first injecting the antimony compounds with the veins occluded and getting no result and then releasing the clamp on the veins and then injecting the antimony salts The results were less satisfactory unless we used large animals as the adrenal

veins frequently became thrombosed after occlusion The general results however, show that these rhythmic movements are due to the output of adrenalin from the adrenal gland which causes an increase in the volume of these organs with marked rhythmic contraction It appears that these powerful contractions of organs like the liver and spleen may bring about a cure by bursting the endothelial cells that are full of parasites and so set them free to be destroyed later by leucocytes One of us (R N C) has utilized these compounds as a provocative test in leishmania infections the results showed that a certain amount of provocative action is present in those cases when parasites are numerous in the spleen After the injections most of the patients complained of a fullness and discomfort in the region of the upper abdomen indicating that there has been an increase in the volume of these organs

(2) *The effect of antimony compounds on the supra renal glands*—The first experiment was done by taking six Belgian hares as controls Six others were given a series of 5 milligrams per kilo body weight of stiburea twice a week for five weeks The first test done was a comparative test, and it showed that the adrenal content of these glands was higher than those in the controls The next experiment was repeated on ten rabbits quantitatively, only five animals were used in each series as one died from an intercurrent disease during treatment The method used for extracting the adrenalin from the glands and obtaining the solution was that advocated by Folin Cannon and Dennis (1913) The actual concentrations of these solutions were measured by giving intravenous injections into a cat following Elliot s method (1912) and comparing the results obtained against a standard solution of Parke Davis adrenalin hydro chloride 1 1000 solution The control rabbits *five in number showed an average adrenal content of 0 311 milligrams* per gramme weight of gland and the average weight of these glands was 0 375 grammes whilst the treated animals showed an average of 0 58 milligrams of adrenalin per gramme weight of gland and the average weight of their glands was 0 366 grammes indicating that the adrenal content of the glands had been nearly doubled compared with that of the normal gland Patients were also tested by Dr J P Bose before and after treatment with these antimony compounds and the table is given below —

It will be seen from these few cases tested that the blood sugar had risen and that the insulin and adrenalin tests were acting as in normal *individuals*

(3) *The effect on the bone marrow*—Dr Napier supplied us with his figures of the leucocyte count before and after treatment with antimony *tartrate In the* cases before treatment the mean works out at 3 000 while the treated cases show a mean of 7 000 leucocytes per cubic millimetre These results were obtained after 30 injections which involved ten weeks treated With the newer compounds Napier has found similar results but the increase in the leucocytes has taken place in four instead of ten weeks We can therefore definitely state that the anti mony compounds increase the leucocytes by stimulating the bone marrow Unfortunately we have not as yet been able to work out *the distribution of*

TABLE II

A. Kala-azar before treatment

Serial No.	Ward	Bed No.	Name	INSULIN RESPONSE		ADRENALIN RESPONSE			Laboratory reports Before admission
				PERCENTAGE FALL OF BLOOD SUGAR AFTER INSULIN		PERCENTAGE RISE OF BLOOD PRESSURE		PERCENTAGE RISE OF BLOOD SUGAR	
				1st hour	2nd hour	¼ hr after	½ hr after	½ hr after	
1	Hatwa	8	Mrs D	12.8	31.4	8.8	8.8	18.8	L. D. bodies Negative
5	Paikpara	10	Ishaque	11.2	19.3	0.0	0.0	7.6	L. D. bodies + Antimony test +++ Aldehyde test
7		6	J T	36.3	33.[illegible]	8.3	3.1	10.0	L. D. bodies +++ Antimony test +++ Aldehyde test +++
9	,,	15	Budhya	66.1	52.3	8.0	8.0	18.6	Peripheral blood not taken Antimony test + Aldehyde test +
11	Hatwa	4	Miss A. J.	20.2	28.5	13.3	0.0	45.3	

TABLE II—*concld*

B *Kala azar after treatment*

Serial No	Ward	Bed No	Name	INSULIN RESPONSE — Percentage fall of blood sugar after insulin		ADRENALIN RESPONSE — Percentage rise of blood pressure		ADRENALIN RESPONSE — Percentage rise of blood sugar	Labratory reports Before admission
				1st hour	2nd hour	¼ hr after	½ hr after	½ hr after	
1	Hatwa	8	Mrs D			1 6	0 0		
5	Pakpara	10	Ishaque	54 1	40 2	22 2	22 2	46 2	
7		6	J T			23 8	17 4	29 4	
9		15	Baidya			19 4	12 4	51 0	
11	Hatwa	4	Miss A J			22 0	16 9	60 9	

antimony in the different tissues of the body nor the rate of its elimination by the urine etc These are two important facts that may throw some further light on the so called resistant cases of kala azar

Discussion

We have seen that leishmania parasites live largely in the reticular endothelial cells of the spleen, bone marrow and liver and rarely in the endothelial tissues situated between the epidermis and corium as seen in post kala azar dermal leishmaniasis In the latter position the leishmania parasites appear to be unable to invade the reticular endothelial tissues of these internal organs In both positions however the parasites are destroyed by the action of antimony but somewhat slower when situated in the skin This seems to show that antimony is capable of stimulating the reticular endothelial tissues and producing substances that destroy these parasites The second mode of action is the stimulation of bone marrow by the increase of leucocytes which cause digestion of the parasites that are free from the endothelial cells This action can be seen to occur in the peripheral blood of any heavily infected case When the bone marrow function is depressed such as occurs when the cases show a leucocyte count of 2 000 or below the parasites run little danger of being destroyed in the peripheral blood but if the bone marrow is too severely damaged the leucocyte count may not return to normal It is probable that the rhythmic contractions of the spleen and liver play a subsidiary part in releasing the parasites from heavily infected cells and allowing them to be destroyed by leucocytes in the circulating blood

The increased function of the adrenals that is caused by these injections produces a marked dilatation of the vessels of the liver and spleen pulp This means an increased permeability of the vascular walls and a diminished permeability in the cells of the reticular endothelium whereby the nutrition of the parasites is affected and multiplication becomes slower and the parasites are finally starved to death After a course of treatment there is also an increase in the blood sugar whether this has any effect on the nutrition of the parasites is at present unknown The resistant cases are not due to faults of the drug but either to the failure of the reticular endothelial tissue the bone marrow or the adrenal glands to respond to these stimuli because they have been damaged by the parasites growing in these tissues or the function of the reticular endothelial tissue has been blocked by the ingestion of protein substances derived from these parasites In dermal leishmaniasis the opposite condition is present the reticular endothelial cells of the spleen and liver are highly resistant to infection by these parasites The blood counts are normal showing that the bone marrow is functionating well and there is generally hypo adrenia also present, the result being that the parasites can only gain a foothold in the reticular endothelium that is situated around the vessels between the corium and epidermis

THE CLASMATOCYTE IN EXPERIMENTAL KALA AZAR

BY

J R CASH

AND

C H HU

Department of Pathology of the Peking Union Medical College, Peking, China

THE studies of Marchand(1, 3), Leishman(2), Ledingham(4), Christophers(5), Laveran(6), Aschoff(7), Meleney(8) and others have solved the question of the ætiology of kala azar and have afforded an excellent description of the anatomical changes produced within the body by this disease. All authors are agreed that *the characteristic change occurring within the tissues of the body is a widely spread* hyperplasia of highly phagocytic cells within which the parasites *Leishmania donovani* are found in great numbers. Due both to their general appearance and their position in the spleen, liver, bone marrow and lymph glands these cells have been generally considered to be of endothelial nature or closely related to endothelium.

There is no anatomical evidence that any of the tissues of the body, other than a number of the heavily parasitized cells just mentioned, are directly damaged by *the parasites of kala azar. The organisms apparently multiply intracellularly* until the cell membrane is ruptured and are then rapidly phagocytized by other nearby or distant cells of similar nature. It is therefore not apparent why the hyperplasia of certain phagocytic, endothelial and wandering cellular elements of the body should be regularly associated with such grave anæmia, leucopœnia and attendant wasting of the tissues as occurs in kala azar.

The anæmia possesses certain unusual features which are not readily explained by the study of the hæmatopoietic system. Though the red blood corpuscles may *be reduced to 500 000 per cubic millimetre, one finds little or no pigmentation of* the tissues with iron, as would be expected were blood undergoing disintegration within the body. Furthermore there are only occasional nucleated red blood cells and young myelocytic leucocytes present in the peripheral blood, thus leading one to believe that little or no effort is being made by the blood forming organs to regenerate new cells. Examination of the bone marrow of the femur from such a case generally affords a great surprise. Instead of the fatty aplastic marrow which one expects, one finds abundant dark grayish red marrow showing marked hyper*plasia of its cellular elements. It is true that* a large part of such hyperplastic

marrow is often composed of the characteristic phagocytic cells of endothelial nature in which the parasites are found There are always however in addition to these larger numbers of marrow cells including nucleated red cells and myelocytes than are present normally in the femur

It therefore seems correct to say that though we are familiar with the direct response of the tissues to *Leishmania donovani* we do not yet know the actual mechanism by which the cells of the peripheral blood are so greatly reduced in number nor do we understand the paradoxical relationship often found to exist between the blood forming tissues and the circulating blood

Our experimental studies of kala azar were begun with the general broad objective in view of attempting to elucidate the fundamental relationship existing between the widely spread cellular reaction shown by the tissues in direct response to the parasites to the striking changes in the blood and blood forming organs

In approaching this problem it seemed to us essential that we should first try to gain a clearer conception than now exists of the exact relationship between *Leishmania donovani* and the cells within which these parasites are regularly found Although they are predominantly present in large phagocytic cells closely related to endothelium in the spleen liver (Kupffer cells) bone marrow and lymph glands these organisms have also been found regularly within phagocytic wandering cells the polymorphonuclear neutrophilic leucocytes of the blood and at times observed in the parenchymatous cells of the liver adrenal and kidney The present paper therefore deals mainly with the nature and distribution of the cells within which *Leishmania donovani* are found

Recent investigations by Sabin and her pupils(9 10 11) W H and M R Lewis(12) Aschoff(13), Maximow(14) Carrel(15) and others dealing mainly with the living cells of the blood hæmatopoietic and connective tissues have suggested a number of important relationships which exist among these tissues and have greatly improved the technique by which their elements may be studied Though considerable difference of opinion still exists regarding most questions related to this general subject it was with suggestions arising from experiments of this kind that our work was begun

Methods

As an experimental animal we have used a hamster *Cricetulus griseus* which is a variety of Chinese field mouse found in great abundance throughout northern China This is the most satisfactory animal which has so far been found for the experimental study of kala azar(16) It is highly susceptible to the disease when the parasites are injected either intraperitoneally or subcutaneously, and tolerates the infection well even though the lesions progress to very advanced stages Moreover these animals are not often attacked by the common laboratory infections of mice and consequently may be kept for long periods of time following infection with kala azar rarely showing any other disease naturally acquired

Throughout these experiments a single strain of *Leishmania donovani* has been used These parasites were obtained in this laboratory five years ago by

transmission of kala azar to hamsters following intraperitoneal inoculation of emulsion made from the spleen at autopsy of a human case of kala azar They have been preserved in highly virulent condition for hamsters by frequent passage through these animals The following routine procedure for transmitting the infection has always been employed in these experiments The enlarged spleen of an animal heavily infected with kala azar was ground well in a mortar, and a suspension of the pulp made by the addition of 15 c cs of normal saline This was centrifugalized for two minutes at comparatively low speed until most of the cellular debris and red blood corpuscles were precipitated leaving a slightly turbid suspension of Leishman Donovan bodies The suspension of parasites was then pipetted off the volume raised to 15 c cs by the addition of normal salt solution, $\frac{1}{2}$ c c of this material which was injected intraperitoneally into each normal animal has never failed to infect hamsters with kala azar

The number of parasites was far from constant in emulsions made from the spleens of different animals but the total dose was always an overwhelming one We have used such large doses only in order to get well marked lesions quickly The animals suffer no direct ill effects from the injection and their spleens generally show early lesions with many parasites within two weeks At the end of three weeks well marked lesions are present at ten to twelve weeks the disease is far advanced

The Peripheral Blood of Hamsters Infected with Kala azar

Films of the peripheral blood supra vitally stained with neutral red and Janus green by Sabin s technique(17) were studied in both normal hamsters and hamsters experimentally infected from three weeks to six months with kala azar The blood was always obtained by puncture of the saphenous vein after removal of the hair and thorough cleaning of the skin with alcohol and ether At the same time blood films were made upon cover slips and stained with Wright s stain The process of supra vital staining for the peripheral blood of hamsters is briefly as follows saturated stock solutions of both neutral red and Janus green are made with absolute alcohol The staining fluid consists of 2 c cs of absolute alcohol to which have been added four drops of neutral red and one drop of Janus green stock solutions Clean flamed slides are flooded with this mixture which is quickly drained back into the bottle to be used over again and then stood on end to dry The alcoholic mixture of dye dries very quickly leaving a thin even film of dye covering the slide The drop of blood to be studied is removed from the bleeding surface on a clean cover slip and immediately placed on the slide covered by the dried coating of dye If the cover slip and slide have been properly cleaned and the dye evenly dried upon the slide the drop of blood quickly spreads out forming an evenly distributed layer of cells The preparation is then ringed with vaseline to prevent drying and placed in a warm microscope box (38°C) where the living stained cells may be studied for about half an hour

Considerable practice is necessary for obtaining a drop of blood from hamsters which is suitable for supra vital study Extreme care must be taken in preparing the skin of the leg which is very tender The foot should be firmly held between the thumb and second finger while a little upward tension is made with the index finger on the skin overlying the vein care being taken not to unduely compress the leg This is necessary in order to keep the puncture wound of the loose skin in apposition with the opening in the vein otherwise the blood escapes into the subcutaneous tissue Needless to say the vein should always be punctured with a very fine sharp needle Slides and cover glasses must be scrupulously clean and all reagents must be of highest purity We have tried many varieties of neutral red and Janus green but have found only those manufactured by Grubler Leipzig and the National Aniline and Chemical Co New York satisfactory Both of these varieties of dye are excellent It is essential that the alcohol used in preparing the stain be free from acid as well as other impurities

We have at times experienced great difficulty in getting the film of stain to dry evenly on properly cleaned slides This occurred only in the rainy season when the great humidity allows so much moisture to be absorbed by the alcoholic mixture of dye with which the slide is flooded that drying takes place very slowly and unevenly Satisfactory films of blood can never be obtained with such unevenly dried slides This difficulty can be readily overcome by preparing the films of dye in a chamber containing artificially dried air or if the humidity is not too great by gently heating the slide as soon as the excess of stain is drained off thereby accelerating the drying process

Nomenclature

We have used the nomenclature adopted by Sabin Cunningham and Doan in their recent studies of the blood and connective tissues The only two terms which might not be fully understood without a word of explanation are those of monocyte ' and clasmatocyte

The term monocyte includes the large mononuclears or transitionals ' of the peripheral blood and cells of apparently the same type which are found in considerable numbers in the spleen lymph glands bone marrow connective tissues These cells are moderately phagocytic for particulate matter and take up colloidal dyes in a specific manner which will be described later on

The clasmatocytes which do not regularly exist in appreciable numbers in the peripheral blood are familiar to all as the macrophages or large wandering phagocytes described by Metchnikoff The term clasmatocyte as used in this paper also includes fixed cells widely distributed throughout many of the organs which have apparently many if not all of the same functions as the wandering cells just mentioned Good examples of this fixed group ' of clasmatocytes are the Kupffer cells of the liver and phagocytic endothelial cells ' of the spleen lymph glands and bone marrow The clasmatocytes in all of these locations react in characteristic manner to certain colloidal dyes

SUPRA VITAL STAINING CHARACTERISTICS OF THE BLOOD CELLS

Although possessing certain peculiar features which will be dealt with in a later study the supra vital staining characteristics of the hamster s blood correspond to those described by Sabin(17) for the blood of humans and a number of other mammals Complete descriptions and illustrations of these may be found in papers by Sabin Cunningham and Doan(9 10 11) It therefore seems adequate to describe in detail only those cells in which Leishman Donovan bodies have been found and certain cells from which they must be differentiated This group is composed of the polymorphonuclear neutrophilic leucocytes the large mononuclears or monocytes and the clasmatocytes

The examination of the peripheral blood consisted of observation of 100 white blood cells in each supra vitally stained preparation Attention was always directed to the condition of the red blood corpuscles The fixed films made from each animal were of great value in substantiating the presence of nucleated red cells and occasional myelocytes the identification of which in supra vitally stained preparations at first caused us considerable difficulty, they were also useful in marking out the distribution of parasites In all the peripheral blood of about 50 hamsters in all stages of kala azar varying from two weeks to five months was examined

The only cells normally present in the peripheral blood in which we actually found *Leishmania* were a *small number of the neutrophilic leucocytes (Plate I* fig 4) These cells were readily identified in supra vitally stained films by their active motility characteristically stained neutrophilic granules which by their size and neutral tint of red may be accurately differentiated from other granules In each hundred white blood cells of heavily infected animals one or two actively motile and otherwise normal looking neutrophilic leucocytes which contained from one to four or five Leishman Donovan bodies were seen The margins and nuclei of these organisms stained at first with neutral red , later on the entire body of the parasite was coloured by the dye We have never observed extra cellular forms in the peripheral blood

The monocytes which normally compose about 1 per cent of the white cells of the hamster s peripheral blood though never seen to contain parasites are in many respects so similar to the parasitized cells seen widely distributed in other tissues that we shall give a short account of them (Plate I, figs 1 2 and 3) They vary from 10 to 15 micra in diameter possess a large eccentric nucleus of oval or indented form which is less dense than the nucleus of the large lymphocytes The cytoplasm is moderately abundant of wavy cloud like appearance and often somewhat vacuolated Situated in the cytoplasm n apposition with the side of an oval nucleus and always in the indentation or Hof ' if this form of nucleus is present is a clear zone around which lies a rosette of neutral red granules These granules are all about of the same size and shade of red Though their colour is not always exactly the same in different cells and is generally darker than the specific salmontint of the monocytes of man and lower animals studied by

Sabin the other characteristics of these cells leave no doubt as to their identity Lying in the cytoplasm grouped more closely around the rosette are a number of mitochondria which stain with Janus green Throughout the rest of the cytoplasm a few masses of neutral red which vary greatly in size may occur these are generally darker than the granules composing the rosette

The peripheral blood of normal hamsters was found to be regularly composed of granular leucocytes monocytes and lymphocytes Frequently a few myelocytes were present, and there were always seen a number of nucleated red blood cells Only on two occasions did we observe clasmatocytes (to be described later) in the blood of normal animals

It may be stated here that the marked anemia and leucopenia regularly observed in human kala azar was not found to occur in hamsters In heavily infected animals the blood appeared to contain myelocytes and nucleated red cells in somewhat greater numbers then normal but no further obvious change was noticed in the other cells

It was quite striking however that clasmatocytes which were only twice seen in normal blood were regularly present in considerable numbers in the peripheral blood of heavily infected hamsters (Plate I fig 5) In the peripheral blood these cells varied from 10 to 20 micra in diameter were round or slightly oval in form Their nuclei which were frequently smaller and more rounded than those of monocytes were eccentrically located in abundant clear often refractile and vacuolated cytoplasm Some of these forms in general morphology resembled the monocytes but their supra vital staining with neutral red allowed their ready differentiation from all other cells Scattered indiscriminately in the cytoplasm were granules and masses of neutral red which varied greatly in size and shade of colour, and were never arranged in rosette formation In very young clasmatocytes a few mitochondria were often present but these tended to disappear in the larger more mature forms

Many of the clasmatocytes in the peripheral blood of hamsters infected with kala azar were observed to contain a few *Leishmania* In very heavily infected animals a few extra cellular parasites were found

Differential counts were at first attempted but were eventually discontinued since the long observations on certain cells frequently rendered it impossible to count the required number of cells while the preparation was still vital le Both the quantitative and qualitative changes taking place in the hamster s blood during kala azar will be dealt with in a further study

SUPRA VITAL STAINING CHARACTERISTICS OF THE CELLS OF THE SPLEEN

The cells of the spleen have been studied in much the same manner as those of the peripheral blood The spleens of 20 normal hamsters and 20 hamsters infected from 10 to 12 weeks with kala azar were examined

The animal to be studied was first anesthetized with ether and the abdominal cavity opened widely exposing the spleen which in infected animals was generally

many times enlarged A clean capillary pipette was plunged directly into the spleen and quickly moved up and down as blood flowed into it This latter procedure was used in order to obtain a large number of splenic cells which must be broken away so that they can enter the pipette along with the blood It was found best to round the edges of the point of the pipette carefully both to prevent too much trauma to the cells as well as blocking of the lumen by large clumps of cells frequently torn away by rough edges A small drop of blood obtained in this way was expressed from the pipette into the centre of a clean flamed cover slip and allowed to spread out upon a slide covered by a dried film of neutral red and *Janus green* * The preparation was then ringed with vaseline and placed in a warm box (37°C) for study From another drop of blood in the pipette films were made upon cover slips and stained with Wright s stain The animal was then killed and the tissues preserved in Zenker s fluid for microscopical study

In both supra vitally stained and fixed films prepared in this manner were found many large clumps of cells which it was impossible to identify There were countless damaged cells masses of reticulum cellular débris and in preparations from infected animals enormous numbers of extra cellular parasites which had been liberated by the trauma of the splenic puncture Sections made from the intact portions of the same spleens showed no extra cellular parasites Since the parasitized cells lie embedded in a meshwork of reticulum from which they are dislodged with difficulty relatively fewer of them appeared in supra vitally stained films than sections showed to be actually present in the spleen Despite these disturbing factors however, numerous intact well stained cells of all varieties including many parasitized cells were available for study

The supra vitally stained cells of the spleen presented a very complex picture which we shall not attempt to analyse here As with the peripheral blood only those cells in which *Leishmania* occur and such forms from which they must be *differentiated* will be considered

In such supra vitally stained films from the spleens of normal animals two distinct groups of large monocytic cells were found(9) (Plate I figs 6 and 7) These were monocytes and clasmatocytes which possessed the same supra vital staining characters as have been described for these elements of the peripheral blood

Freshly made preparations from infected hamsters showed clearly that the clasmatocytes were greatly increased in both number and size of the individual cells Furthermore aside from rare neutrophilic leucocytes each containing only a few parasites (Plate I fig 12) it was only within the clasmatocytes that Leishman Donovan bodies were found The clasmatocytes varied from small forms not much larger than a red blood cell to huge cells reaching as much as 60 micra in diameter (Plate I figs 8 9 10 and 11) These large varieties often

* For the study of the cells of the spleen the mixture of dye should be twice the strength of that used for the peripheral blood

were packed with parasites to such an extent that the nucleus was no longer visible Though forms so distended with *Leishmania* had usually lost their ability to take up neutral red to such an extent that certain examples could not be recognized as clasmatocytes, characteristically staining intermediate varieties, within which parasites were less numerous left little doubt as to their identity

We have examined 1 000 cells of supra vitally stained preparations made from each of the spleens of 20 normal hamsters and 20 hamsters infected from 10 to 12 weeks with kala azar These observations included only intact forms not obscured by other cells or débris Though most of the clasmatocytes which are very fragile, were either ruptured in making the preparation or were present in large clumps along with other cells which partly obscured them and were therefore not counted, the relative number of clasmatocytes was nevertheless found to be greatly increased (Table I) No significant change was noted in the relative number of monocytes

TABLE I

Number of animals	Total cells of spleen counted	Monocytes		Clasmatocytes	
		Total number	Percentage	Total number	Percentage
20 normal	20 000	340	1 7	214	1 0
20 infected with kala azar (2 to 20 weeks)	20 000	368	1 84	1 242	6 21

We should here like to call attention to the small number of monocytes which we have found in the spleens of both normal and infected hamsters Since other observers(11) have found from ten to twenty per cent of the splenic cells in human and a number of lower animals to be monocytes and there is moreover, considerable evidence to show that these cells are to a large extent formed in the spleen we feel that further attention should be given to the identification of the monocytes in hamsters We can now only say that typical monocytes of all ages occurred in the spleens of our hamsters in the numbers stated in Table I The clasmatocytes were universally characteristic, and every mononuclear cell in which parasites were found was clearly a clasmatocyte

PHAGOCYTOSIS OF *Leishmania donovani* 'IN VITRO' BY NEUTROPHILIC LEUCOCYTES

Due to their motility irregular shape and characteristic staining qualities, it was quite easy to pick out very quickly a number of neutrophilic leucocytes in such preparations of the spleen even though they were present in relatively small numbers

As has been stated in freshly made films from the spleen of animals infected with kala azar, one only occasionally found a neutrophilic leucocyte containing a

few *Leishmania* Upon allowing such a preparation to remain in the warm microscope box for half an hour, we were surprised to find that practically every neutrophilic leucocyte had become packed with parasites

It was obvious that this phagocytosis of organisms had taken place on the slide, for upon constant observation of single cells for a few minutes after the preparations were made we were soon able to see actively motile neutrophilic leucocytes engulf extra cellular *Leishmania* which were generally so abundant (Plate I fig 12) Such cells appeared to suffer no direct ill effects from the parasites taken into their cytoplasm, retaining their motility and supra vital staining for about the same length of time as do the neutrophiles of normal animals

Further proof that this phagocytosis of parasites took place after the cells were removed from the body was afforded by fixed films of the splenic cells, and sections of the spleens A thorough search in such preparations from the same animals revealed only very rare polymorphonuclear leucocytes containing *Leishmania*

Histological Study of the Tissues of Hamsters with Kala azar after Intravenous Injection of India Ink

Although study of the supra vitally stained parasitized cells left little doubt as to their identity as clasmatocytes it must be said that the method employed had the disadvantage of not allowing the study of all the cells of the spleen *in situ* The criticism might well be made that certain either very fragile or firmly attached cells of other varieties which contained parasites, though existing in the spleen in considerable numbers might not have been present in our supra vitally stained preparations

Since it is known that the clasmatocytes are the most avidly phagocytic cells of the body for particulate matter, it was decided to study sections of the tissues of animals which had first been infected with kala azar, and later on injected intravenously with a small amount of India ink If all of the large parasitized cells proved to be clasmatocytes they should contain ink granules as well Any appreciable number of cells in which *Leishmania* were present, but which did not phagocytize ink, would soon become apparent The method possessed the added advantage of allowing the study of all the tissues of the body

Observations were first made upon a series of 8 hamsters which 16 days following inoculation with kala azar, were each injected intravenously with 0 2 c c of India ink During the following 7 days these animals were studied Supra vitally stained films were first made from the spleen , the animal was then killed, and the tissues fixed in Zenker's fluid Sections were stained with hæmatoxylin and eosin, and Giemsa's stain All of these animals were found to show early, widely spread lesions of kala azar

After thorough study, it was clearly seen, in both supra-vitally stained films of the spleen and sections of the various organs, that all of the large cells, in which

parasites were seen contained numbers of ink granules as well (Plate I, figs 14 15 and 16) As would be expected the amount of ink in many of these cells was often so great that a few parasites which might have been present were totally obscured This was not a very disturbing factor however for fixed films stained with Wright's stain practically always revealed intra cellular *Leishmania* regardless of the amount of ink present within the same cell Supra vital staining of the cells of the spleen showed a few monocytes and an occasional lymphocyte which contained a few scattered granules of ink but parasites were never found in such cells

Though this distribution of the parasites in early lesions of kala azar was quite clear we felt that the possibility existed of a somewhat different relationship in the more advanced and chronic cases of the disease In the attempt to clarify this point we first injected each of a series of 10 normal hamsters with 0 2 c c of India ink and 2 days later inoculated them intra peritoneally with kala azar These animals were studied in the same manner as the first series 28 to 49 days after inoculation of the parasites All of them showed typical very advanced lesions of kala azar

The results of observations upon both supra vitally stained films from the spleen and sections of the principal organs were practically identical with those obtained from the study of the series of animals with early infections All of the parasitized cells even the largest ones which were distended with a hundred or more organisms contained ink as well In the animals of this series due to the wide distribution of a small amount of ink among an infinitely greater number of clasmatocytes than were present in the series of early infections parasites were rarely if ever hidden from view in any of the preparations by ink granules The amount of ink present within individual cells varied greatly Sections showed clearly that this was due in a large part at least to the positions of the cells Parasitized cells which were grouped in large masses generally contained fewer granules of ink than isolated forms or those lining blood channels This fact could readily be accounted for by position alone since those forms which were heavily loaded with ink were always found in locations where they would have ready and frequent access to granules of ink liberated into the blood stream In addition to this physical explanation we have also the distinct impression that younger forms of clasmatocytes, and those which contain relatively few parasites have much greater powers of phagocytosis than the older forms especially those forming large masses which are generally greatly distended with *Leishmania* The supra vitally stained films of the spleen showed that the monocytes and lymphocytes contained no ink as would be expected so long after injection

Phagocytosis of ink granules by the cells forming the characteristic lesions in kala azar is illustrated by Plate II These two series of photomicrographs were taken from two hamsters in about the same stage of the disease Figs 1 3 and 5 show typical moderately advanced lesions in the spleen liver and bone marrow Figs 2 4 and 6 illustrate corresponding lesions in an animal which had been

injected intravenously with India ink just before inoculation with *Leishmania donovani*

Leishmania donovani IN THE SKIN AND SUBCUTANEOUS TISSUE OF HAMSTERS INFECTED WITH KALA AZAR

In none of our hamsters infected with kala azar now numbering somewhat over one thousand have we ever observed any gross alteration of the skin Even after the hair has been removed it has never been possible to detect any change of colour or texture of the skin over such an area

Since however the skin and subcutaneous tissue are tissues in which considerable numbers of *clasmatocytes are known to exist under normal conditions* we considered it of importance to determine whether or not *Leishmania donovani* were present within the clasmatocytes of the skin and subcutaneous tissue of hamsters infected with kala azar

Very dilute normal saline solution of neutral red was injected subcutaneously *until the tissues were œdematous* After a few minutes a small bit of subcutaneous tissue was removed and spread out upon a warm slide mounted in a drop of the neutral red saline under a cover glass ringed with vaseline and studied in a warmed microscope box in the same manner as the supra vitally stained films of blood cells A glance at our first preparation of this kind revealed great numbers of typical clasmatocytes which were filled with *Leishmania* (Plate I fig 18) This has *been a regular finding in practically all animals after the infection has reached a* certain intensity We have found the infection to proceed with about equal rapidity in all the members of a series of hamsters experimentally infected from the same suspension of parasites In studying the individual members of such a series at weekly intervals it has been found that the skin and subcutaneous tissue of practically all of them become parasitized at about the same time One series may reach this point within a month while six or eight months may at times be necessary for such a distribution of parasites to occur

were

but

possessed all of the supra ... cells

They were found to ingest India ink in large amounts if injected subcutaneously but we have so far never found ink granules in these extra vascular clasmatocytes in instances when this substance was injected intravenously (Plate I fig 19) Examination of the subcutaneous tissue from all areas of the body revealed a very even *distribution of parasitized cells*

Upon studying the distribution of these parasitized cells in sections of the skin and subcutaneous tissue they were found to be most numerous about the blood vessels from whose walls they frequently seemed to arise In many instances a thick layer of such cells a number of which were *undergoing mitosis* were *found* to surround completely practically all of the small vessels Many parasitized cells were frequently found around the sweat glands and scattered diffusely throughout

all levels of the corium and subcutaneous tissue Numerous lymphocytes a few larger mononuclear cells which were probably monocytes and plasma cells were generally found among the accumulations of clasmatocytes No disturbances of pigmentation or changes in the hair follicles were seen

Discussion

The two principal facts brought out by these experiments are the identification of the large mononuclear phagocytic cells in which *Leishmania donovani* are found as clasmatocytes and the presence of huge numbers of such parasitized cells in the skin and subcutaneous tissue of hamsters experimentally infected with kala azar

It has been obvious to most observers of both cutaneous Leishmaniasis and kala azar that great similarity existed between the parasitized endothelial cells' of the viscera and the wandering phagocytic cells of the connective tissue At least two authors Versari(19) and Meleney(8) have expressed this view by designating as clasmatocytes' all of these large cells in which the parasites are found It has not been altogether clear from their observations however that the parasites were limited in their distribution to the specific cells which the name clasmatocyte' implies In sections or fixed films upon which all previous observations of the tissues in kala azar have been made one has been confronted by a massive number of parasitized cells which individually vary greatly in size shape and staining qualities The inference that all of these forms belong to the same group has been made due to certain non specific staining characteristics and the observation that many of the parasitized cells are markedly phagocytic for red blood corpuscles, pigment and cellular debris as are the wandering cells of connective tissue

Both the fixed and wandering mononuclear phagocytes have been objects of intensive study by numerous investigators during recent years Mallory(21) who was among the first to deal with this subject proposed the term endothelial leucocyte for the cells of this type This conception now widely accepted has been subjected to much experimental study

One school perhaps led by Aschoff has directed its attention mainly to the ability of these cells to deal with particulate matter and certain colloids injected into the blood stream Aschoff concludes that there are two varieties of mono nuclear phagocytes The first type which is derived from specialized endothelium widely distributed throughout the body but mainly situated in the spleen liver lymph glands and bone marrow may remain fixed at their sites of formation as do the Kupffer cells of the liver or may wander widely as macro phages' The second type composed of the large mononuclears of the blood and similar cells found largely in the same situations as the strictly endothelial phagocytes of the first type are supposed to arise from reticular cells very closely related to endothelium These two types of phagocytes Aschoff finds so closely related in appearance origin and function that he has grouped them together as a physiological unit under the name of the reticulo endothelial system Since the introduction of this very helpful conception of the phagocytes of this type

a number of their functions have been brought to light but considerable difference of opinion still exists among investigators as to the origin and functional identity of the two types of cells originally grouped together by Aschoff to form this so called system

By the use of certain colloidal dyes Sabin and her co workers have studied the vital and supra vital staining characteristics of these two groups of cells Their observations have led them to conclude that the monocytic phagocytes are composed of two distinct types of cells the clasmatocytes which arise from specialized endothelium and the monocytes which arise from reticular cells widely distributed in the tissues The large mononuclears of the blood are considered by them to be monocytes Upon studying these two types of cells under a number of pathological conditions they conclude that the two varieties should not be grouped together as a functional unit The reticulo endothelial system of Aschoff accordingly would then include the endothelial system ' composed of fixed and wandering endothelial phagocytes and the 'reticular system composed of monocytes only

Our experiments with kala azar have shown that only those cells which have the supra vital staining reactions of clasmatocytes compose the very characteristic group of phagocytic mononuclear cells in which the parasites causing this disease are found In an earlier preliminary communication(20) we have spoken of kala azar in hamsters as a *disease of the reticulo endothelial system* at the same time pointing out that the small number of cells of the hamster which we could identify as monocytes apparently played no role in the disease process Since we have found that supra vital staining reveals only insignificant numbers of monocytes in the spleen we realize that more experimentation upon hamsters especially directed to the study of the monocytes is necessary before we from our own experience can take a definite stand upon the relationship existing between these two types of cells which present such different reactions to vital dyes We have no hesitancy however in stating that the large mononuclears which normally compose about one per cent of the leucocytes of the peripheral blood are fundamentally different from the parasitized phagocytic cells The monocytes were never observed to contain parasites nor to be obviously increased in number in any of the animals infected with kala azar On the whole the weight of evidence so far gained from the study of experimental kala azar supports the conception that the two types of phagocytic cells revealed by supra vital staining are of distinctly different origin and under certain *conditions at least possess different functions* This view is based only upon the specific relationship apparent between *Leishmania donovani* and only those mononuclear phagocytes which stain supra vitally as clasmatocytes

From our observation of rapid phagocytosis of parasites by neutrophilic leucocytes in vitro ' one might infer that such cells act as defensive agents against these organisms The finding of occasional neutrophiles in the peripheral blood and other tissues which contain a few *Leishmania* is in accord with such an idea

Due to the comparative infrequency with which such cells are found in cases of kala azar, one is certainly justified in concluding that the parasites do not multiply within the neutrophilic leucocytes

Leishmania donovani have been found by numerous observers within the parenchymatous cells of the liver adrenal and kidney in both human cases of kala azar and experimental animals This has also been true in hamsters but occurred only in very heavy infections As a rule the number of parasites found within each of such cells was very few in comparison with the average number within the individual clasmatocytes Only in rare instances where the tissues were swarming with *Leishmania* did it ever seem likely that the parasites were multiplying within parenchymatous cells Due to the fact that inert matter such as India ink may have the same distribution such findings have not been considered as worthy evidence against the conception of a specific relationship between *Leishmania donovani* and the clasmatocytes The parasites are distributed apparently in almost the same manner as the granules of ink but only those which lodge in the clasmatocytes multiply We say almost in the same manner as ink only because we have never seen these organisms within the monocytes Since the number of cells which we have identified as monocytes has been found so limited in hamsters it is quite possible that a few parasites resting within an occasional cell of this type as granules of ink frequently do might have escaped observation

With this conception of the relation existing between *Leishmania donovani* and the tissues of the body we may say that these parasites may invade or be phagocytized by a number of varieties of cells but that their multiplication and general distribution commonly is specifically associated with the clasmatocytes only Under conditions of overwhelming infection it is possible to conceive of further alterations between the parasites and cells of the host which allow multiplication of parasites in other situations such as the cells of the liver

The presence of large numbers of parasitized clasmatocytes in the skin and subcutaneous tissue of hamsters infected with kala azar is a matter of considerable interest One naturally suspects that this condition arose in large part at least due to contamination of the subcutaneous tissue with parasites during intraperitoneal inoculation There are a number of reasons for not holding this view In the first place a small number of parasites such as would be expected to escape into the superficial tissues during intraperitoneal inoculation if directly injected into the subcutaneous tissue only occasionally produce infection with kala azar or survive *in situ* in sufficient numbers to allow their detection at a *subsequent date Secondly the distribution of parasitized cells in both light and* heavy infections of the superficial tissues is a very even one over the entire body, indicating that invasion by parasites occurred from a number of sources, rather than spread from the original puncture wound of the abdominal wall Thirdly, we have demonstrated the same lesions in human cases of kala azar(18)

Due to the limited amount of human material at our disposal, we have no grounds for estimating the relative importance of the skin and subcutaneous tissues

as sources from which kala azar may be transmitted If it is demonstrated however that human cases ever show the degree of infection of these tissues as we have regularly found in heavily infected hamsters such findings must be taken into account in further work upon the natural transmission of the disease

We should also like to call attention to observations not yet complete bearing upon the question of origin of the wandering phagocytes We have frequently found small blood vessels in these tissues completely surrounded by a thick mantle of parasitized cells which have been found to possess the same supra vital staining characteristics as the clasmatocytes of normal subcutaneous tissue and parasitized clasmatocytes of the viscera Frequently such groups of cells show numerous mitotic figures and individual cells may be found which are partially connected by cytoplasmic processes to the adventitia of blood vessels Though quite aware that such observations do not furnish positive proof that these cells may arise extravascularly they at least support such a view A number of parasitized cells seen throughout the skin and subcutaneous tissue and among the fibres of skeletal muscle when studied in sections bear a close resemblance to fibroblasts They are greatly elongated possess long branching processes and generally contain fewer parasites than the other more numerous forms Since we have not found parasitized cells in vitally stained preparations of connective tissue which do not show all the characteristics of clasmatocytes we are still of the opinion that all of the parasitized cells of the superficial tissues are of the same variety We are, however making further studies of these forms which in sections have the morphological characteristics of fibroblasts

CONCLUSIONS

A specific relationship has been demonstrated in the hamster between *Leishmania donovani* and the clasmatocytes of both fixed and wandering types

Attention has been called to the great numbers of parasitized clasmatocytes regularly found in the skin and subcutaneous tissue of hamsters heavily infected with kala azar

Points brought out by these experiments which bear upon the morphological characters function and origin of the phagocytic mononuclear cells have been discussed

REFERENCES

(1) MARCHAND F (1903) Zur Kenntnis der sogenannten Bantischen Krankheit und der Anæmia Splenica *Munch Med Woch* No 11 S 463

(2) LEISHMAN W B (1903) On the Possibility of the Occurrence of Trypanosomiasis in India *Brit Med Jour* Vol I p 1252

(3) MARCHAND I and LEDINGHAM J C G (1904) Uber Infection mit Leishmanischen Korperchen (Kala azar ?) und ihr Verhältniss zur Trypanosomen Krankheit *Zeitschr f Hyg u Infekt onskrank* Vol CCCCLXXV S 1

(4) *H* (1904) On the Quest on of Trypano oma Inf t n n Man *Lancet* Vol I p 149

(5) CHRISTOPHERS S P (1904) On a Paras te f n l in Persons suffe n from enlarge ment of the Spleen in In l a S ent fic Memoirs of the Covernment of In lia New Ser es No 11

(6) LAVERAN A (191) Le hmai o es Par s Masson

(7) ASCHOFF L (1904) Den onstrat on von Präparaten e nes Falles von Kala azar *Ve han ll ngen ler deuts hen p thologschen Ge ell haft* S 8

(8) MELENEY H E (19 5) The H stopatl ology of Kala azar n the Han ster Monkey and Man *An er Jo r of Path* Vol I p 147

(9) SABIN F R D AN C A anl C NNINGHAM I S (1 25) I s r m nat on of Two Types of Pha yt c Cells n the Co nect ve Tis ues by S pra v tal Techn que Con tr but o s to E bryology Car eg e Inst tute of Wash n ton Vol XVI No 8 p 125 (Goo l B bl ography)

(10) DOAN C A C NNINGHA I S anl SABIN F I (19 5) Exper n ental Stu l es n the Or g n and Maturat on of Av an and Mammal an R d Pl od Cells Contr bu t o s to Eml ryolo *Carne e Inst tute of Wash n ton* Vol XVI N 83 p 163 (Coo l B bliography

(11) CUNNINGHAM I S SAB N F P and DOAN C A (19) The D elopment f Le o yte I ympl o ytes an l M nocytes from a Spe f Ste C ll n Ad ult T ss es C t r b t ns t Embryol y Carne Inst tute of Wa h ngto Vol XVI No 84 p 7 (Good B bl o raphy)

(12) LEWI M R anl W H (19 5) Th Tran form at o f Wl l d C l *Jour Inr Mel Iss* Vol LXXXIV p 8

(13) ASCHOFF L KIYONO K (1913) Zur Frage ler gro e Mo o u l aren *F d Hæm tolog* Vol XV p 383

(14) MAXIMOW A (19 3) Untersu hung n uler Blut un l I ndegewebe *Arcf f n kr Anat* Vol LXXIII S 444

(15) CARREY A anl FEELING A H (1926) Fun lan ental Propert es of E brobla t an l Ma rop age *Jour Exp Med* Vol XLIV p 61

(16) YOUNG C W SMYLY H J anl BROWN C (1924) Exper mental Kala aza n a Hamster *Proc Soc Exp B ol and Med* Vol XXI p 357

(17) SABIN F I (1923) Stud s on Liv ng Hu nan Bl o l Cell *Jol ns Hopk ns Hosp B ll* Vol XXXIV p 77

(18) CASH J I anl HU C H (19 7) Demonstrat on of *Le h nan a dono n* n the Sk n and Subcutaneo s T ssu of Kala azar Patients *Jour Amer Med A oc* I Pr s

(19) VERSARI A (19 6) Un caso l buttone l Oriente n Provinc a d Napol *La Ped a* a Vol XXXIV No 18 p 977

(20) HU C H and CASH J I (19 7) Cons derat ons upon the Relat onsl p of the Ret ulo E dothel al System to Kala azar *Proc Soc Exp B ol anl Med* Vol XXIV p 469

(21) MALLORY I B (1898) A H stological Study of Typho d Fever *Jour Exp Med* Vol III p 611

PLATE I

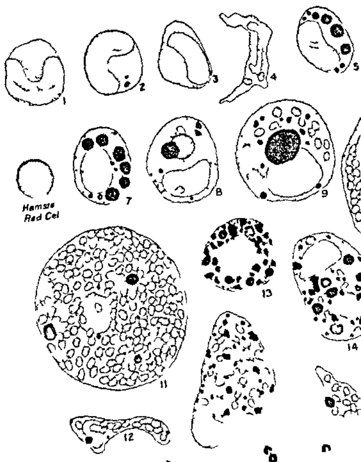




Fig. 1.



Fig. 2.

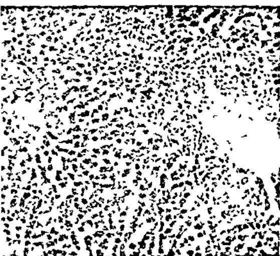


Fig. 3



Fig 4.





Explanation of Plate II.

Figs 1 3 and 5 Photomicrographs ×120 Spleen liver and vertebral bone marrow of hamster 10 weeks after inoculation with kala azar showing moderately severe lesions of this disease Attention is called to the characteristic distribution of the lesions which consist of marked hyperplasia of large phagocytic cells (clasmatocytes) about the periphery and in the centre of the lymphoid follicles in the spleen within both the peripheral and central zones of the liver lobule and scattered widely throughout the bone marrow The granular appearance of these cells is due to the presence of Leishman Donovan bodies which are too small to be clearly visible at this magnification

,, 2 4 and 6 Photomicrographs × 120 Spleen liver and vertebral bone marrow of hamster 8 weeks after inoculation with kala azar This animal received an intravenous injection of 0 2 c c of India ink two days before inoculation with kala azar The areas from which these photographs were taken correspond to those shown in Figs 1 3 and 5 All of the parasitized cells were found to contain ink granules In Fig 4 granules of ink are shown in the liver cells

STUDIES UPON THE PERIPHERAL BLOOD, BONE MARROW AND SPLEEN OF HAMSTERS EXPERIMENTALLY INFECTED WITH KALA AZAR

BY

C. H. HU

AND

J. R. CASH

Department of Pathology of the Peking Union Medical College, Peking, China

Although Chinese hamsters are highly susceptible to kala azar if inoculated with *Leishmania donovani* and show typical lesions which have appeared to a number of observers as strictly comparable to those seen in human cases of kala azar, the general health of these animals is very slightly affected by the disease. We have no statistics upon the average span of life in normal hamsters and those infected with kala azar, but the fact that many of these animals with heavy infections survive in apparent good health under laboratory conditions for as long as two years is sufficient proof that kala azar pursues quite a different course in hamsters from that regularly observed in human beings. When sacrificed for study at any time following inoculation with parasites, the subcutaneous fat has always been found abundant and there never occurred any obvious wasting of the skeletal muscles. Though we have taken precaution to protect our animals from other diseases which might be acquired in the laboratory, it has been interesting to note that those infected with kala azar have succumbed to intercurrent infections in about the same numbers as normal hamsters. The total number of this group has been very small in both cases. It has been quite striking, however, that the infected group as a whole has withstood both excessively warm and cold weather less well than the normal animals. We have made no observations other than

which causes such typical and severe lesions of the viscera of experimental animals fails to produce in them the same severe symptoms by which kala azar is manifested in human cases.

By a casual glance at a blood film made from a heavily infected hamster one sees that no very marked anæmia or leucopænia is present We are now bringing forth evidence which shows that this is actually the case and in our opinion sheds some light at least upon the manner in which such extensive reduction of the blood cells takes place in human beings affected with this disease

Procedures and Methods

Total cell counts of the peripheral blood and differential counts of the cells of the supra vitally stained peripheral blood bone marrow and spleen have been made upon normal hamsters and hamsters infected from one to seven months with kala azar The infection was always transmitted by intraperitoneal inoculation of ½ c c of an emulsion made from the spleen of a heavily infected animal as we have previously described(1)

The drawing of blood from Chinese hamsters in quantities sufficient for both a red and white count presented a number of difficulties These animals whose average weight is about twenty five grammes possess no veins well adapted to bleeding The saphenous vein the only vessel which can be used for this purpose is covered by very loosely attached skin which slips easily over its surface The leg should first be scrupulously cleaned and the skin overlying the vein held taut by a little upward traction If the vein is then carefully punctured with a very fine sharp round needle which is introduced at right angles to its surface bleeding generally takes place freely

We have also made differential leucocyte counts of the peripheral blood and the cells of the bone marrow and spleen supra vitally stained with neutral red and Janus green This technique and the criteria for identifying the various types of cells of the blood and hæmatopoietic tissues have been completely described by Sabin and her co workers in several beautifully illustrated monographs(2 3 4) We may say briefly that the method consists in allowing a drop of blood to spread out beneath a well cleaned cover slip on a slide covered by a thin dried coating of dye(1) The preparation is then ringed with vaseline to prevent drying and studied with the microscope in a box warmed to body temperature The cells of the bone marrow and spleen were obtained by puncture of these structures with capillary pipettes while the animals were anæsthetized We have always made control films on cover slips which were stained with Wright s stain These have been very useful in identifying unusual forms of myelocytes and confirming our observations upon nucleated red cells

The Peripheral Blood

The red corpuscles and leucocytes of the peripheral blood have been counted in fourteen normal hamsters and fifty hamsters experimentally infected from one to seven months with kala azar

The following three tables show the results of these observations —

TABLE I

Cell Count of Peripheral Blood of Normal Hamsters

Serial No	R B C per c mm	W B C per c mm
1699	11 2[illegible]0 000	1[illegible] 060
1700	1[illegible] 090 000	19 740
1701	11 530 000	13 380
1702	10 56[illegible] 000	4 860
1703	12 8[illegible]5 000	9 830
1704	10 700 000	6 650
1707	12 481 500	10 9[illegible]0
1708	8 981 500	8 175
1709	9 700 000	6 660
171[illegible]	10 [illegible]65 625	9 600
1713	9 725 000	6 520
171[illegible]	12 050 000	8 760
1716	9 970 000	10 100
1717	10 481 500	9 780
Average (14 animals)	10 8[illegible]9 000	9 789

TABLE II

Cell Count of Peripheral Blood of Hamsters Infected with Kala a ar (Early stage 35 to 43 days after inoculation)

Serial No	Duration of Infection	R B C per c mm	W B C per c mm
1577	35 days	9 0 000	16 7 0
1578	35	6 675 000	7 3 0
1579	37	10 370,000	5 800
1580	37	6 830 000	11 560
1584	37	9 0 000	5 930
1585	38	7 530 000	8 240
1586	38	8 950 000	7 00
1587	41	10 300 000	6 040
1588	41	9 0 000	3 100
1589	41	10 500 000	6 1 0
1 9	4	9 160 000	8 140
159	43	9 1 0 000	15 0 0
159	4	10 830 000	3 580
1	43	6 7 0 000	14 150
1596	43	10 150 000	10 435
Average (15 animals)		8 976 000	8 6 3

TABLE III

Cell Count of Peripheral Blood of Hamsters Infected with Kala azar (Late stage 132 to 210 days after inoculation)

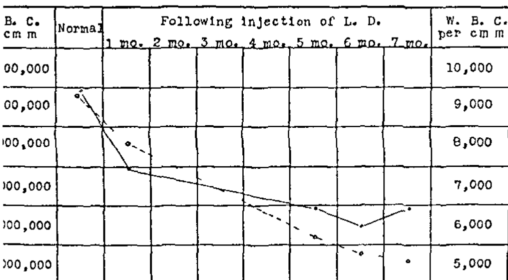
Serial No	Duration of Infection	R B C per c mm	W B C per c mm
1041	13 days	10 0 0 000	100
10 3	13	8 950 000	6 840
1024	13	8 185 000	3 5 0
1028	13	6 00 000	4 900
1030	133	9 150 000	13 5 0
1039	134	8 7 0 000	7 780

TABLE III—*concld*

Serial No	Duration of Infection	R B C per c mm	W B C per c mm
738	136 days	9 931 000	4 300
927	137	8 137 500	3 720
998	138	8 400 000	5 0[illegible]0
996	139	7 080 000	6 840
9[illegible]4	139	7 512 500	4 470
1000	140	4 650 000	4 480
1004	140	9 700 000	9 940
713	146	5 650 000	5 990
566	147	6 975 000	11 740
819	149	8 450 000	4 060
803	176	8 400 000	7 780
804	177	4 745 000	4 700
805	178	5 495 000	6 990
806	179	8 750 000	4 160
807	180	5 755 000	2 920
808	180	9 362 500	2 900
800	181	10 181 500	10 980
763	200	9 [illegible]87 500	7 500
768	201	5 137 500	4 200
769	201	8 662 500	5 640
742	208	10 500 000	3 900
750	208	7 570 000	4 000
769	208	8 881 000	6 260
773	208	7 480 000	6 520
740	209	6 970 000	4 820
741	209	8 875 000	4 860
774	209	6 850 000	6 280
776	209	8 790 000	6 780
744	210	6 970 000	5 670
Average (35 animals)		7 858 000	5 900

CHART

Cell Count of Peripheral Blood



The results of all of these cell counts which are summarized in the Chart show that moderate anæmia and leucopænia occur in hamsters infected with kala azar The most rapid reduction in red corpuscles took place during the first month following inoculation with parasites after which time they continued to be reduced in number until the fifth month but later on showed no significant change The number of leucocytes declined gradually for the entire period of seven months

We have also counted the red and white cells of the blood of a number of hamsters heavily infected with kala azar at intervals up to two years following inoculation with parasites but have never found anæmia or leucopænia appreciably greater than demonstrated by the systematic counts recorded in the Chart

After following the quantitative changes in the cells of the blood for seven months it seemed clear that the hamster was in some way protected from the severe anæmia and leucopænia which regularly develop in human cases of kala azar The fact that a moderate reduction of both red corpuscles and leucocytes did occur in hamsters infected with this disease indicated in our opinion that the course of kala azar in hamsters is fundamentally the same as in human beings although the point at which clinical symptoms appear is never reached It was obvious that this did not depend upon the degree of infection or the numbers of parasites

present, for both the typical visceral lesions and parasites in hamsters three months after inoculation with *Leishmania donovani* were generally far more numerous than seen in human cases of kala azar

The problem was now to determine why anæmia and leucopænia did not develop to marked degree in hamsters Differential leucocyte counts were next done upon blood films supravitally stained with neutral red and Janus green of normal hamsters and hamsters heavily infected with kala azar These are recorded in Tables IV and V

TABLE IV

Differential Cell Count of the Peripheral Blood in Normal Hamsters

Animal No	1129	1131	1139	1148	1147	1160	1161	1162	1165	1163	Average per cent
Total cells counted	200	200	200	200	100	200	100	300	200	200	
	Per cent.	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
Neutrophilic leucocytes	8 5	17 5	19	10 5	7	7 5	16	6	17 5	15	12 45
Eosinophilic leucocytes	4	8	2	2	5	1 5	3	7	2 5	2 5	3 75
Lymphocytes (small)	82 5	39 5	69	80 5	83	75	69	77	69 5	69	70 7
Lymphocytes (large)	5	34 5	15 5	6	5	13 5	11	9	10	13	12 25
Monocytes	0	0	0 5	1	0	1 5	1	1	0 5	0	0 55
Myelocytes	0	0 5	1	0	0	0 5	0	0	0	0 5	0 [illegible]
Clasmatocytes	0	0	0	0	0	0	0	0	0	0	0
Unclassified	0	0	0	0	0	0 5	0	0	0	0	0 05
Nucleated red cells per 100 leucocytes	0	0	0	0	1	1 5	2	1	2 5	0	0 8

TABLE V

Differential Cell Count of the Peripheral Blood in Hamsters Infected with Kala azar
(Four months after inoculation with Leishmania donovani)

nal No	1032	1035	1036	1037	1038	1041	104?	996	998	1000	1004	100?	Average per cent
l cells unted	200	200	200	200	200	100	200	200	200	200	200	200	
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
ph lic ocytes	29	26 5	17	19 5	16	11 5	10	15	10 5	14	13 5	13	16 3
pl lhc ocytes	2	2	0	0 5	0	1	0	0	0·5	0	0	0 5	0 54
nocytes ll)	33	46	45 5	36 5	51 5	48	64	55	54 5	53	59 5	60 5	50
nocytes ge	4 5	8 5	14	5	3	7	5	5	1 5	6	4	7 5	5 9
ytes	0	0	0	0	0	1	0 5	0	0	0	0	0	0 12
ytes *	9	4 5	2 5	10 5	6 5	7	2	6	6	1	3	4	5 2
ytes	1	1	3	3 5	0 5	2	2 5	4	1	4 5	0 5	1	2
stocytes	21 5	11 5	16	24 5	19	23	16	15	26 5	21 5	19	13	18 8
sified	0	0	2	0	1	0	0	0	0	0	0 5	0 5	0 03
ted red per 100 ocytes	2	0 5	1	3	4	0	1 5	0	3 5	0 5	1 5	0	1 46

The principal change in the peripheral blood of hamsters infected with kala azar has been found to consist in the appearance of a large number of clasmatocytes Many of these cells contained parasites In addition to this it became clear that both myeloid activity and erythrogenesis were increased Increased numbers of myelocytes nucleated and reticulated red cells were seen in the blood of the infected animals

The monocytes presented certain difficulties in identification which will necessitate further experimentation for solution We have listed as monocytes only typical forms and have placed the doubtful ones which appeared only in infected animals in a separate group These latter cells we have not been able to distinguish from certain myelocytes and unparasitized clasmatocytes

The analysis of the cells of the bone marrow supra vitally stained with neutral red and Janus green showed a number of changes in the group of hamsters infected with kala azar The results of these counts are given in Tables VI and VII The

leucocytes their predecessors and clasmatocytes were counted differentially, and the number of nucleated red cells seen during the counting of these cells recorded The fact that so many cells, especially the very fragile clasmatocytes were destroyed or badly damaged in making supra vitally stained preparations of bone marrow, seriously interfered with the accuracy of the method It must be understood therefore that the figures in the table are not meant to represent the actual cell content of the marrow, but merely indicate the kind of change which takes place in kala azar

TABLE VI.

Differential Cell Count of the Bone marrow in Normal Hamsters

Animal No	1104	1105	1150	1152	1153	1134	1135	1136	1137	1138	Average per cent
Total cells counted	200	200	200	500	400	400	300	400	300	400	
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
Neutrophilic leucocytes	61	53.5	70.5	75.5	73	53	65	70	66	70	66
Eosinophilic leucocytes	4.5	0.5	0	0.4	1.7	5.3	2.7	0.5	2.4	4	2.3
Lymphocytes	0.5	5.5	1	0.9	0.3	1	3.1	1.5	2.3	2	1.8
Monocytes	0	0	0	0	0	0	0	0	0	0	0
Clasmatocytes	1.5	7.5	3	0.5	1	3	1.7	1.5	1	1.5	2.2
Myelocytes A	1.5	2	0.5	0.6	0.3	1	0.3	1	1.3	0.5	0.9
Myelocytes B	6	6	6	2	2.2	3.5	1.3	3	2	2	3.4
Myelocytes C	3	6	1.5	1	4.2	9.5	6	6	2.3	3.5	4.3
Myelocytes, Mis	21	18.4	17	18.5	14.6	22.7	17.3	11	20	14.5	17.5
Unclassified	1	2	1	1	3	1	3	7	3	2	2.4
Nucleated red cells per 100 leucocytes	80	85	71	53	76	116	105	91	174	55	91

As in the peripheral blood, a distinct increase of the clasmatocytes in the bone-marrow of hamsters, infected with kala azar, was noted This was much greater than the records show, for most of the clasmatocytes from infected animals were ruptured in removing the cells from the marrow cavity of the femur, and were therefore not counted Large fragments of these damaged cells were seen scattered throughout the preparations It was also clear that the number of clasmatocytes

TABLE VII

Differential Cell Count of the Bone marrow in Hamsters Infected with Kala azar.
(Four months after inoculation with Leishmania donovani)

Animal No	1010	1011	1012	1014	1015	1016	1018	1020	1021	1022	Average per cent
Total cells counted	300	200	400	400	400	400	400	400	400	400	
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
Neutrophilic leucocytes	54	54.5	66	61	60	57	60	52	67.5	51	58
Eosinophilic leucocytes	2.3	2.5	6.7	1	0.75	1	0.75	0.5	1.5	0.75	1.8
Lymphocytes	0	0	0	0	0.5	1.2	4.5	2	1	1	0.8
Monocytes	0	0	0	0	0	0	0	0	0	0	0
Clasmatocytes	4	7.5	6	2	3	3	1	8	4	4	4.25
Myelocytes A	0.3	3	0	0.3	0.5	0.5	4.8	0.3	0.5	2	0.9
Myelocytes B	1.3	6	1.2	1.5	1.3	1.3	3.8	1.8	1.5	0	2
Myelocytes C	3.3	4.5	2	1.5	2.3	2.5	1	4.3	1.3	1.5	2.5
Myelocytes Ms	31.3	19.5	17.4	31.5	25.6	26	28	28	22.5	32	26
Unclassified	4	3	1	1	6	7	0	4	1	7	3.4
Nucleated red cells per 100 leucocytes	91	105	121	38	57	57	100	114	68	83	84

destroyed in removing marrow cells was much greater in infected than in normal animals This was in part due to the greater number of clasmatocytes present in infected animals but also in great measure to the fact that the large parasitized clasmatocytes are much more fragile than normal cells of this type

We should like to call attention to certain peculiarities of the myelocytes of hamsters We have arbitrarily arranged the myelocytes in four different groups There was no doubt as to the correct identification of the cells listed as myelocytes C and miscellaneous myelocytes Those forms listed as myelocytes A and B may be monocytes of which we have found no typical examples in the bone marrow of either normal or infected hamsters Since the numbers of these two latter groups of cells were found to be so small in normal animals and to show no significant variation after inoculation with kala azar they have only little bearing upon the present question We shall not devote space to their description at present, but merely state that here, again, we have encountered difficulties in identifying

monocytes in hamsters Since typical examples of these cells undoubtedly exist in the hamster s peripheral blood the exceedingly small number of monocytes found in the spleen and bone marrow are facts worthy of comment It is our opinion that a special study of the monocytes in hamsters may be of considerable value in determining the origin of these cells

A considerable increase in the relative number of myelocytes was noted in the bone marrow of the animals infected with kala azar This was in accordance with the findings in the peripheral blood

The number of nucleated red cells was apparently reduced in the infected animals but not in sufficient degree to indicate any impairment to erythrogenesis On the other hand it was clear that blood formation was proceeding more rapidly than normal in the bone marow of hamsters infected with kala azar for the number of erythroblasts was found to be about twice that of the marrow of normal animals which showed predominance of erythrocytes

The spleen was found much more suitable than the bone marrow for differential counts of its supra vitally stained cells Though our studies of the bone marrow of hamsters had suggested an increase in the rate of blood formation after infection with kala azar we were by no means sure that this was the case until the spleen

TABLE VIII

Differential Cell Count of the Spleen in Normal Hamsters

Animal No	11?9	1131	1139	1147	1148	1160	1161	1162	1163	116?	Average per cent
Total cells counted	400	300	400	400	300	300	200	400	400	400	
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
Neutrophilic leucocytes	5 5	8 5	3 5	9 5	5	[illegible]	5	4 5	6 5	17	6 7
Eosinophilic leucocytes	0 5	1	0	[illegible]	0 7	0 7	0	5 5	0 8	2 5	1 4
Lymphocytes	88	81	91	81	88	91	81	84	80	69	83 4
Monocytes	0	0	0	0 5	0	0 3	0	0	0	0 5	0 1
Monocytes ?	0	0 7	1	0 5	0	1 3	0 5	0	0	0	0 4
Clasmatocytes	2 5	1 5	1 5	1 5	5	3	2	0 5	2 5	3 5	2 3
Myelocytes	0	1	0	0 5	0	0	0	1	0	1	0·35
Unclassified	3 5	6 3	3	5	1 3	2 2	11 5	4 5	10 5	6 5	5 4
Nucleated red cells per 100 leucocytes	8	18	17	18 5	8	29	25 5	6	26 5	24	18

was examined with this point in view Tables VIII and IX give the results of differential counts upon the supra vitally stained cells of the spleen together with observations upon the relative number of nucleated red blood cells present in the spleens of normal animals and following infection with kala azar

TABLE IX

Differential Cell Count of the Spleen in Hamsters Infected with Kala azar
(Four months after inoculation with Leishmania donovani)

Animal No	1032	1035	1036	1037	1041	996	998	1000	1004	1005	Average per cent
Total cells counted	400	400	400	400	400	300	300	400	400	400	
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	
Neutrophilic leucocytes	8 5	7	5	5	6 5	5 7	23 6	12	11 5	8	9 3
Eosinophilic leucocytes	0	0	0	0	1	0 7	0	0 25	0	0 25	0 2
Lymphocytes	60	64 5	79	58 5	59 5	50 3	47	38 5	47	61 5	56 6
Monocytes	0	0	0	0	0	0	0	0	0	0	0
Monocytes ?	0	0	0	1 3	0 5	0 3	1 3	1	0	0	0 4
Clasmatocytes	18	14	11 5	19	18	19	14	30	28	25	19 7
Myelocytes	4	9 5	9 5	3	5 5	4 6	6 7	2 5	0 5	1	3 8
Unclassified	9 5	5	4	12	9	19	7	1[illegible]	13	3	9 4
Nucleated red cells per 100 leucocytes	78	37	70	54	99	67	76	46	90	75	69

We were quite surprised to find the evidence of such marked blood formation in the spleens of normal hamsters The cell counts of the spleens of animals infected with kala azar showed clearly one immediate reason why hamsters do not develop marked anæmia and leucopænia after infection with this disease As shown in the tables the myelocytes were increased to about ten times their normal number while the nucleated red corpuscles of the spleen were present in number comparable to that of all other splenic cells excluding the erythrocytes as 2 to 3 The clasmatocytes as expected were greatly increased in the spleens of the infected animals These changes may be seen beautifully in sections of the spleens Plate III figs 1 and 2 illustrates the extensive myeloid reaction which occurs in the hamster s spleen after infection with kala azar As in the peripheral blood and bone marrow we have had some doubts as to the classification of certain cells

resembling monocytes Such cells which have been tabulated as questionable *monocytes* have been found in such small numbers in the spleens of both normal and infected hamsters that they have little bearing upon the anæmia and leucopænia in hamsters infected with kala azar The often rather large group of unclassified cells included reticular cells and a few forms which were probably myelocytes but were *composed mainly of a group of very characteristic cells vet unidentified* These forms varied from about ten to twenty five micra in diameter were generally of oval or slightly irregular shape and possessed small round or oval nuclei Their cytoplasm which was quite abundant was generally filled with refractile vacuoles No part of *these cells ever stained with either neutral* red or Janus green A few refractile granules were frequently seen in the cytoplasm Such cells appeared to be more numerous in the spleens of infected animals but were never observed to contain parasites

Discussion

These observations have shown that only moderate degrees of anæmia and leucopænia develop in the hamster following experimental infection with kala azar *The most rapid reduction in number of the red corpuscles was found to occur* within the first month after intraperitoneal injection of parasites After this time the anæmia progressed more slowly but never reached a degree of severity comparable to that regularly seen in human cases of kala azar

The fact that anæmia and leucopænia did definitely appear regularly in hamsters infected with kala azar has been taken to indicate that the disease process is fundamentally the same in hamsters and man But the observation that the degrees of these changes shown by hamsters are relatively slight has furnished evidence that infection with kala azar is dealt with by the hamster in a manner somewhat different from that shown by human beings affected by this disease It was also incidentally rendered clear why the general health of hamsters is so slightly altered by heavy infection with *Leishmania donovani*

Differential counts of the supra vitally stained cells of the peripheral blood and bone marrow have given evidence of increased myeloid activity and erythrogenesis in hamsters infected with kala azar This fact was shown most clearly, however by the differential counts made upon the cells of the spleen It was found that the spleen of normal hamsters is the site of considerable blood formation but that after infection with kala azar the numbers of both nucleated red cells and myelocytes in this organ were enormously increased This observation made first upon supra vitally stained films of the splenic cells has been confirmed by fixed films stained with Wright s blood stain and by sections We have not yet completely studied in sections the morphological changes *in the spleen after* infection with kala azar

Though the scope of these studies is limited to the explanation of the mild clinical course which kala azar pursues in hamsters certain suggestions have arisen which touch upon the *ætiology of the marked anæmia in human* cases of the disease

In attempting to explain the manner in which anæmia in man is caused by kala azar the actual destruction of red corpuscles already in the circulation may be ruled out both upon the lack of any direct evidence that this is the case as well as the striking absence of pigmentation of the tissues It is however to be borne in mind that unusually large numbers of red cells are probably removed from the circulation through phagocytosis by the clasmatocytes which are enormously increased throughout the body This fact is hardly adequate to entirely explain the anæmia since it does not account for the reduction in hæmatopoiesis shown by the picture of the peripheral blood It seems therefore that some marked impairment to blood formation must occur in human beings infected with kala azar which is not present in hamsters affected with the same disease

The obvious impairment to the formation of blood which is common to both man and hamsters is the marked hyperplasia of clasmatocytes which takes place in the marrow cavities of the bones These cells are often so numerous and show such marked evidence of rapid multiplication that pictures comparable to those produced by malignant tumours replacing the bone marrow occur The hamster responds to this situation by active extra medullary formation of blood which is not shown by man to any great degree under similar conditions We have observed one case of human kala azar however in which the inner table of bone of the skull had been eroded with marked extension of actively hyperplastic myeloid and erythrogenic tissue to the outer surface of the dura It therefore seems that if the bone marrow escapes from the confines of its cavities the formation of blood may take place This is not a very usual occurrence The striking degree to which extra medullary blood formation may occur in man is too well known to require comment The reason for its absence in kala azar must therefore lie in some fundamental impairment of this activity produced by the disease

The experiments of Sabin and her associates have shown that the red blood cells arise intra vascularly from endothelium lining the collapsed capillaries of the bone marrow from which they escape when mature(3) The clasmatocytes of both fixed and wandering types have been found by these investigators to be likewise of endothelial origin

In view of the close genetic relationship that has been shown to exist between the red blood cells and the clasmatocytes it is therefore not surprising that alterations in the blood should occur in such a disease as kala azar which affords such extreme stimulation to the formation of clasmatocytes In this disease the endothelium, lining the sinuses of the spleen liver lymph glands bone marrow engages in such active proliferation in the formation of cells of the clasmatocyte type that it is probable that any potentialities which such endothelium may have for the formation of blood are not manifested Such a view is in accordance with the facts now in hand and is not seriously opposed by the fact that extensive extra medullary formation of blood takes place in hamsters infected with kala azar

It is of course not to be expected that human compensating for such marked damage to the bone m by the formation of blood in other parts of the body further limit their ability to do so These observatio in explanation of the anæmia in human cases but as fu relationship existing between the clasmatocytes and a blood forming potentialities of endothelium in those characteristic lesions of kala azar are found Such lo which extra medullary formation of blood would be severe anæmias

CONCLUSIONS

It has been shown that hamsters infected with kal degrees of anæmia and leucopænia

This is largely due to extensive extra medullary been studied in the spleen but which probably occurs

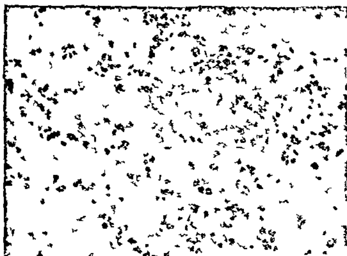
REFERENCES

(1) CASH J R and HU C H (1927)	The Clasmatocyte in I *tions of the 7th Co*
(2) SABIN F R DOAN C A and CUNNINGHAM R S (1925)	Discrimination of Tw the Connective T Contributions to Em Washington Vol bibliography)
(3) DOAN C A CUNNINGHAM R S and SABIN F R (1925)	Experimental Studies Avian and Mamm to Embryology C Vol XVI No 83 p
(4) CUNNINGHAM R S SABIN F R and DOAN C A (1925)	The Development of Monocytes from Tissues Contributi Institute of Washin (Good bibliography)

PLATE III



Fig 1 Photom rograph (930) of fro en se t on of spleen of no mal han ste Th kness of sec on m a ta ned w th Good pasture s oxy lase sta n to show prese ce of m lo d lls



Fg 2 Photom crograph × 930) of fr ze sect n f sp een of hamster three months after noculat on w th kala azar Th ckne s of s ct on 15 m cra Goo l pasture s oxydase sta n

THE PRESENCE OF *LEISHMANIA DONOVANI* IN THE SKIN AND SUBCUTANEOUS TISSUE IN CASES OF KALA AZAR

BY

J R CASH

AND

C. H HU,

Department of Pathology of the Peking Union Medical College, Peking China

ATTENTION has already been called to the occurrence of enormous numbers of clasmatocytes filled with Leishman Donovan bodies in the skin and subcutaneous tissue of Chinese hamsters experimentally infected with kala azar(1) That these same changes may be present in human beings who have acquired the infection naturally has been shown by the demonstration advanced of lesions identical with those of hamsters in the skin and subcutaneous tissue of one fatal case of kala azar studied at autopsy and of a smaller number of parasitized cells in the subcutaneous tissue of another similar case(2)

During the past year we have succeeded in securing specimens of skin removed from ten living patients suffering with kala azar who have been studied in the hospital of the Peking Union Medical College In each instance the skin was fixed in Zenker's fluid and sections prepared which were stained with hæmatoxylin and eosin When the size of the specimen was sufficient, some of the subcutaneous tissue was cut into small bits and injected intraperitoneally into hamsters

All of these patients have been proven to have kala azar by the presence of Leishman Donovan bodies in smears made from the blood removed by splenic puncture None of them had ever received any treatment for kala azar previous to the removal of skin The results of these studies are summarized in the following table

Case 2 though parasites were very numerous in the spleen, showed light infection of the superficial tissues of the body *Case* 8, in which parasites in the spleen were few revealed very many parasitized cells in the skin and subcutaneous tissue It is interesting to note that both positive cases showed infection of both pieces of skin which were removed from widely distant areas

We have already published a short description of these lesions to which there is nothing essential to be added now We should like to repeat, however, that a

Results of examination of sections of skin and subcutaneous tissue removed from patients infected with kala azar

No	Age	Duration	L D in films splenic puncture	L D in sections of skin and subcutaneous tissue
1*	14	6 months	++++	Chest—negative, arm—negative
2	14	1 year	++++	Chest—positive, arm—positive
3	14	2 years	+	Chest—negative, leg—negative
4	20	3 years	+	Chest—negative, thigh—negative neck—negative
5	24	1 year	++	Arm—negative, leg—negative
6	6	1 month	+	Arm—negative, leg—negative
7	4	4 months	+	Arm—negative, leg—negative
8	10	2 years	+	Chest—positive thigh—positive
9	20	1½ years	+	Chest—negative, thigh—negative
10	5	1 year	+	Chest—negative thigh—negative

* Typical kala azar was produced in hamster examined three months after intraperitoneal injection of finely cut bits of subcutaneous tissue from this case

distinct increase of mononuclear cells about the blood vessels has been a constant finding in all of the cases of the present series Many of these cellular forms in the subcutaneous tissue have been shown to be clasmatocytes by supra vital staining with neutral red

The number of cases in this series is too few to allow any generalization concerning the extent to which parasitized cells may be present in the superficial tissues of human cases of kala azar, but does suffice to demonstrate that parasites may be present in considerable numbers without causing any obvious change in the skin Neither of the positive cases showed any discoloration of the skin, though several of those in whose skin no parasites were found showed moderate pigmentation, mainly of the face, arms and trunk

Most of these cases, which we have had the opportunity of examining have been of very chronic type, and, as indicated in the table, have shown only a few parasites in the spleen Though adequate for demonstration of the principles involved such cases do not afford suitable material for studying the degree to which

the superficial tissues of the body may be filled with parasites It is obvious that more acute and active cases must be studied before the importance of the skin and subcutaneous tissue as foci from which the infection is naturally distributed, is established

REFERENCES

(1) CASH, J R, and HU, C H (1927) The Clasmatocyte in Experimental Kala azar *Transactions of the 7th Congress F E A T M* Vol III

(2) *Idem* (1927) Demonstration of *Leishmania donovani* in the Skin and Subcutaneous Tissue of Kala azar Patients *Jour Amer Med Assoc* (In Press)

tissues were found to be in an unusually good state of preservation, due to refrigeration of the body, we do not think that the apparent absence of parasites was due to delay in performing the autopsy

The spleen, which weighed 660 grammes, was of the same leathery consistency and showed micro scopical changes quite analogous to those seen in Case 1 Considerably more numerous nucleated red blood cells and myelocytes however were present A few megakaryocytes were also seen A small infarct due to thrombosis of an arteriole was found No Leishman Donovan bodies were present though a number of large phagocytic cells were seen in the sinuses *The liver* was of pale, yellowish brown colour and rather soft Microscopical examination showed nothing other than the presence of considerable fat within the liver cells in the portal areas The Kupffer cells were not altered No Leishman Donovan bodies were found The *bone marrow* of the femur, ribs bodies of the lumbar vertebræ and calvarium was of firm consistency and dark red colour Microscopically the marrow, all of these areas showed the same type of extensive hyperplasia as did the bone marrow of Case 1 Upon stripping the dura away from the calvarium changes identical in nature with those seen in the first case were found The inner table of bone presented the same honeycombed appearance and was adherent on both sides in the frontal and parietal regions to soft, friable, grayish red tissue covering corresponding areas of the outer surface of the dura Microscopical examination showed that the inner table of bone had been eroded and bone marrow widely formed on the surface of the dura Plate IV, fig 2, shows the gross appearance of the interior of the skull The dura presented the same picture illustrated in fig 3, while sections made of the skull and dura were identical with those shown in *Plate V*, figs 4 and 5 *Plate V, fig 8, illustrates the marked erythrogenic and myelocytic activity of* the extra medullary bone marrow on the dural surface No Leishman Donovan bodies were found in any of the preparations of bone marrow

The cervical lymph nodes showed moderate hyperplasia associated with the infection of the mouth and neck The remaining lymph glands brain, lungs, kidneys adrenals pancreas, intestine, gall bladder, thymus, testis and skin showed no significant changes

Emulsions of the *spleen*, liver and bone marrow of femur and dura were injected into hamsters, but none of the animals developed kala azar

Discussion

We have been confronted with two very similar instances of children with marked anæmia and leucopænia associated with unusually extensive, widely spread hyperplasia of the bone marrow It is very surprising that the peripheral blood in neither case showed any evidence of this greatly increased hæmatopoietic activity The first case was proven to be kala azar by the demonstration of Leishman Donovan bodies in extra medullary bone marrow, while the second case, as suggested by the typical clinical picture, globulin precipitation test and similar findings at autopsy, was in all likelihood of the same ætiology, though no parasites were ever demonstrated during life, at autopsy, or by animal inoculation Since the autopsy on the second case was not performed until four days after death, a small number of parasites which might have been present were probably no longer viable at the time animals were inoculated

Both of these cases, assuming that the second was also one of kala-azar, illustrate the fact that the degree of anæmia and leucopænia occurring in chronic cases of this disease do not always directly depend upon the number of parasites in the body, or the presence of the usual characteristic hyperplasia of clasmatocytes The diagnosis of kala azar was made certain only by the demonstration of a comparatively small number of Leishman Donovan bodies in

the first case The other changes though compatible with this diagnosis were not specifically characteristic of kala azar

It is difficult to explain the unique situation presented by aplastic blood picture hyperplastic bone marrow and lack of evidence such as pigmentation of the tissues pointing to the undue destruction of blood within the body It is obvious that the blood forming organs were making a supreme effort to supply the deficiency in blood cells While it is conceivable that changes not obvious in the complex picture presented by the bone marrow of these cases might result in marked impairment of its functional capacity, it is difficult to understand why no nucleated red cells or myelocytes were found in the peripheral blood We see no theory by which this can be accounted for other than the assumption that these young forms were in some way prevented from entering the circulation Just what the nature of such an obstruction might have been is not clear but it seems likely that extensive hyperplasia of endothelium in the formation clasmatocytes which in all likelihood took place earlier in the disease caused alterations in the relationship of the capillaries and blood sinuses of the bone marrow which have prevented the escape of maturing blood cells

One cannot be sure that such formation of clasmatocytes ever occurred in either of these two cases but in view of the constant anatomical changes produced by kala azar it is very probable that such was the case This change however which characteristically takes place in the spleen liver lymph glands bone marrow and to less extent elsewhere had to a large extent retrogressed in the two cases under discussion Only in the bone marrow of the skull and that formed on the dura were any appreciable numbers of clasmatocytes found

The hæmatopoietic activity shown by the spleen in both cases was distinctly greater than usually seen in kala azar although the anæmia and leucopænia were not as severe as frequently occurs This finding is in accord with our previous suggestion that extra medullary blood formation is restricted in cases of kala azar(1) Its occurrence to such degree in these two cases could be explained by the absence of the usual widely spread lesions of this disease

Summary

Two unusual almost identical cases one definitely proven to be kala azar and the other by analogy of the same ætiology have been described A maximum degree of generalized hyperplasia of the bone marrow was found in both cases which had led to erosion of the inner table of the skull and extensive extra medullary blood formation on the surface of the dura The absence of nucleated red corpuscles and myelocytes in the peripheral blood in the presence of such marked hæmatopoietic activity was accounted for by the theory that such forms were not able to escape in sufficient numbers from their sites of formation The likelihood of such an obstruction due to earlier changes in the bone marrow which had largely retrogressed has been pointed out The specific lesions of kala azar



Fig 1



Fig. 2.

Explanation of Plate IV

Fig 1 Skull from Case 1 showing bilateral erosion of inner table of bone in frontal regions

,, 2 Skull from Case 2 showing similar erosion of inner table of bone in right parietal region

,, 3 Dura from Case 1, showing extensive formation of bone marrow on outer surface

Explanation of Plate V

Fig 4 Photomicrograph (× 30) of lesion of skull in Case 1, showing erosion of inner table of bone

" 5 Photomicrograph (× 30) of dura in Case 1 showing large masses of bone marrow on the outer surface The thickening of the inner surface of the dura is also shown

" 6 Photomicrograph (× 230) of frozen section of bone marrow taken from surface of dura in Case 1 Goodpasture's oxydase stain to show myeloid cells

" 7 Photomicrograph (× 1000) of bone marrow from surface of dura in Case 1 Stained with hæmatoxylin and eosin A few clasmatocytes filled with Leishman Donovan bodies are shown

" 8 Photomicrograph (× 1000) of bone marrow from surface of dura in Case 2 No parasites were found in this tissue, which in all other respects was similar to the extra medullary marrow of Case 1



Fig. 4.

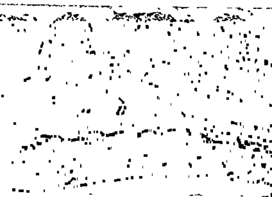
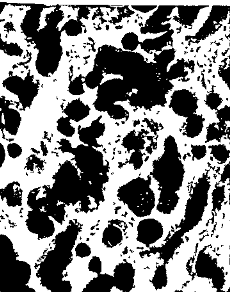
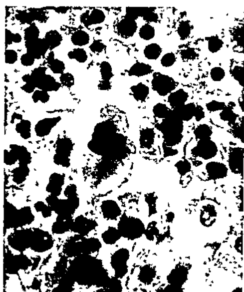


Fig 5.



Fig 6





PERIPHERAL LESIONS PRODUCED BY *LEISHMANIA DONOVANI* AND ALLIED ORGANISMS *

BY

CHARLES W. YOUNG

AND

MARSHALL HERTIG

Department of Medicine, Peking Union Medical College, Peking, China

GONDER(1), Laveran(2, 3) and Sergent(4) have reported peculiar lesions of the extremities, tail, scrotum, nose and ears in white mice inoculated intraperitoneally with recently isolated cultures of *L. tropica*. Sergent was unable to produce such lesions with cultures beyond the fiftieth cultural passage.

Brahmachari(5) and others in Calcutta have reported cases of kala azar treated with antimony and apparently cured, in whom there developed later nodules in the skin of the face, upper extremities and trunk, in which Leishmaniæ were found and from which they were cultivated. The spleen, liver and blood stream in these cases were free from Leishmania. Brahmachari has called this complex dermal leishmanoid. Acton and Knowles(6) reported a patient diagnosed clinically as xanthoma tuberosum multiplex. A Leishmania was cultivated from the lesions.

Nicolle(7) in Tunis has had under artificial cultivation on NNN medium for many years strains of the organisms from local human kala azar, canine kala azar and from the gecko.

Experimental.—Intraperitoneal inoculation of Acton and Knowles' "xanthoma" strain into Chinese striped hamsters (*Cricetulus griseus*) has produced only visceral lesions. The same is true of cultures from a case of "dermal leishmanoid." The flagellates of both of these strains were agglutinated by the sera of rabbits immunized against Indian strains of *L. donovani*. From these reactions it may be assumed that these organisms are *Leishmania donovani*.

The cultures obtained from Tunis have acted in a different and peculiar manner. We have used strains K A and Sh of *L. donovani* (*L. infantum*, "kala azar humain"), strains x and X of *L. canis* ("kala azar canin") and strain G 6 of *L. tarentolæ* ("leptomonas de gecko") from the gecko. All of these strains originally produced

* Assisted by grants from the China Medical Board of the Rockefeller Foundation.

visceral lesions only When these cultures were inoculated intraperitoneally into hamsters, the infections were visceral at first, with enlarged spleen and liver and with Leishmaniæ fairly abundant in the smears from the spleen, liver, bone marrow and heart blood After a lapse of from two months to over a year from the time of inoculation bilaterally symmetrical lesions began to appear, in the following order (1) swellings of the carpi and tarsi extending later to the feet including the digits, (2) swelling of the posterior half of the scrotum in males, with subsequent ulceration (infiltration and enlargement of the clitoris, exceptionally with ulceration of the perineum in the female), (3) swelling (infiltration) and later ulceration of the base of the tail, (4) similar swelling of the nose, rarely with ulceration, and (5) swelling and ulceration of the margins of the ears From the swollen tissues, Leishmaniæ enclosed in large mononuclear cells (clasmatocytes) were obtained, often in large numbers The lesions of the feet never showed ulceration The clasmatocytes were present often in enormous numbers between the connective tissue fibres of the ligaments of the carpi and tarsi and distally (feet including digits) The cells containing parasites in the lesions showing ulceration, were in the deep layers of the skin and subcutaneous tissues Intraperitoneal inoculation of the tissues from these peripheral lesions produced the same picture in two to four months and have continued to do so consistently and repeatedly The same pathological changes were obtained with all the five strains used As the peripheral lesions developed, those of the viscera tended to disappear, so that at autopsy some of the animals had normal sized spleens and livers, negative for *Leishmaniæ* in smear Intraperitoneal inoculation of such tissue did not cause infection in hamsters Thus it will be seen that these five strains which originally produced visceral lesions only, now cause pathological changes precisely like those described by Gonder, Laveran and Sergent for recently isolated *L tropica* Similar lesions of a single extremity were found in two hamsters inoculated with two different Chinese strains of *L donovani* These hamsters, already showing heavy visceral infection had been tied out with leather thongs constricting the four extremities during insect feeding experiments several months before the appearance of the lesions Inoculation of tissue from these local swellings produced only general visceral infections like those caused by the strains with which these hamsters had been originally infected

We acknowledge, with thanks, the courtesies of Drs Nicolle and Anderson of Tunis and of Dr Knowles and Dr Napier of Calcutta in supplying cultures

REFERENCES

(1) GONDER (1913) .. *Arch f Sch u Trop Hyg*, Vol XVII, p 397
(2) LAVERAN (1914) .. *Comp Rend Acad Sci*, Vol CLIX, p 539
(3) *Idem* (1915) .. *Bull Soc Path Exot*, Vol VIII, p 363
(4) SERGENT (1926) .. *Ann Inst Past*, Vol XL p 411
(5) BRAHMACHARI (1922) .. *Ind Med Gaz*, Vol LVII, p 125
(6) ACTON and KNOWLES (1925) .. Annual Report of the Calcutta School of Tropical Medicine, Calcutta, India
(7) NICOLLE (1925) .. *Arch Inst Past*, Tunis Vol XIV p 136

DIAGNOSTIC VALUE OF THE ANTIMONY TEST IN KALA-AZAR

BY

LIEUT COL R N CHOPRA, M A, M D (Cantab), I M S
J C GUPTA, M B (Cal)

AND

N. K BASU, M B (Cal)

Department of Pharmacology Calcutta School of Tropical Medicine and Hygiene

In the *Indian Medical Gazette* for June 1927 we described a new test for the diagnosis of kala azar This test was based on the fact that when solutions of organic compounds of antimony were brought in contact with the serum from kala azar patients a thick flocculent precipitate was produced at the junction of the two * This precipitate was not produced with normal sera or when sera of patients suffering from most other diseases were tested in a similar way Most of the organic antimonials reacted in this manner though the precipitate produced by some of them was much more marked than that formed by the others With the urea derivatives of antimony the precipitate produced was immediate and striking and as these compounds are commonly used for the treatment of visceral Leishmaniasis in this country and are readily available we recommended their use for the test As regards the strength of the solution required we found that though 0 05 per cent or even weaker solutions formed a precipitate it was advantageous to use 1 to 4 per cent solutions of the antimony compounds The precipitate thrown down by the weaker solutions was not so dense flocculent and distinctive as with the stronger solutions These latter give a thick flocculent

* *Technique* —The serum separated from the blood is pipetted out in a capillary tube with a rubber teat and is put into a small test tube made by cutting ordinary glass tubing 3 to 4 mm in diameter 3 to 3½ inches in length and sealed at one end A 4 per cent solution of one of the organic compounds of antimony—preferably a urea compound such as urea stibamine—is slowly added along the side of this tube (with a capillary pipette) With kala azar serum a thick flocculent precipitate forms at once while with the other sera there is either no precipitate whatever or only a slight precipitate appears at the junction of the two The flocculent character of the precipitate is more clearly seen if test tubes of under 5 mm diameter are used than larger ones and our standard of precipitate formation is based on following this technique

precipitate which shows a tendency to conglomerate into a mass at the junction of the two fluids and is difficult to break by shaking Further, the precipitate does not dissolve if the tubes are allowed to stand for 24 hours or longer Antimonyl tartrates as a rule gave no precipitate

It is interesting to note in this connection that besides the organic compounds of antimony a number of other compounds form a precipitate with the kala azar serum but this characteristic reaction of the antimonials is not obtained The organic compounds of arsenic such as sulfarsenol produce a well marked precipitate but this does not show a flocculent character and besides the precipitation occurs with non kala azar sera also Further the precipitate formed by the arsenicals is completely dissolved if the tube is allowed to stand for a few hours The colloidal solutions of gold also formed a precipitate or turbidity with kala azar serum but a similar reaction is obtained with sera from chronic malaria cases By observations on a large number of sera we found that this reaction of organic antimonials could be used for differentiating kala azar from non kala azar sera and therefore, could be used as a diagnostic test for the disease

We further endeavoured to simplify this test so that it could be performed by any practitioner at the patient s bed side This was done by eliminating the process of removal of the blood from the vein and the separation of the serum from the corpuscles which takes time We succeeded in substituting the whole blood obtained from a finger prick for the serum in the test The technique was described in the *Indian Medical Gazette* for August 1927 and consists in receiving two or three drops of blood in ¼ to ½ c c of a 2 per cent solution of potassium oxalate (2 per cent potassium citrate or normal saline may be used) A little of this is transferred to a glass tubing 3 to 4 mm in diameter sealed at one end by a capillary pipette with a rubber teat The solution of an organic compound of antimony (such as urea stibamine) is then added and this being heavier sinks to the bottom Almost immediately a flocculent precipitate appears and slowly settles down in a characteristic manner resembling a shower of rain It is better to let the red blood corpuscles settle down before testing the supernatent fluid if this is not done the results are not so clear As a rule the characteristic flocculent precipitate is fully developed in 3 to 5 minutes If the tubes are left longer even non kala azar blood may produce haziness or granular precipitate which may be misleading

In very early cases of kala azar the precipitate may take a minute or two before it appears but in well developed cases it forms immediately and flocculation is quite distinct We have not had the opportunity of doing the blood test extensively but from the results we have obtained so far this test if carefully done appears to be of even more value than the serum test Often non kala azar sera which give a doubtful reaction with the serum test are negative with the blood test Since these tests were described, we have been able to apply them to a large number of sera from both kala azar and non kala azar patients with a view to confirm their diagnostic

value in the disease and our results have been corroborated by independent observers A lot more work is necessary before the efficacy of these tests is fully established but from the work already done the tests appear to have certain advantages over the commonly used aldehyde test of Napier in the diagnosis of kala azar

The standard of precipitate formation

Before giving the results of our observations it will be necessary to give an idea as to the standard of precipitate formation we have adopted for the test in which the serum separated from the blood is used to be considered as positive A slight precipitate or even haziness may sometimes form with certain sera and unless trouble is taken to differentiate this carefully from the thick flocculent precipitate met with when the test is positive a wrong diagnosis may be made The test is now being largely used and we have learnt that the technique described by us is not being followed and not infrequently sera have been returned as positive whenever any sort of precipitate is produced by bringing the antimony solution in contact with the serum We therefore wish to lay particular stress on the fact that a flocculent appearance of the precipitate is of prime importance in the reaction and unless flocculi are unmistakably present the test should not be considered as positive We have also found that the flocculent character can be more clearly seen if small test tubes of 3 to 4 mm in diameter are used than larger ones For the purpose of this reaction all sera come under four main categories —(1) Firstly strongly positive sera (+++) that is sera from those cases in which the disease is well advanced The precipitate here is thick flocculent forms immediately and conglomerates into a thick mass at the junction of the serum and the antimony solution This precipitate does not dissolve on standing for 24 hours and is difficult to break on shaking (2) Secondly positive sera (+) i e the sera which give a definite flocculent precipitate which conglomerates into a mass is insoluble on standing but is not so well developed and thick as the first group Patients coming under these two groups give a characteristic precipitate with the blood test also (3) Thirdly doubtful sera (±) which give a definite precipitate or in some cases varying degree of haziness appear at the junction of the two fluids If the precipitate after careful observation does not show signs of flocculation it is generally non kala azar if however there are signs of it and if the precipitate does not dissolve on standing it is strongly suspicious of the serum coming from an early case of kala azar A precipitate is not uncommonly formed with sera obtained from patients suffering from diseases in which there is marked disturbance of metabolism For example chronic malaria with enlarged spleen leukæmia advanced tuberculosis leprosy syphilis etc occasionally form a precipitate but this precipitate may not show definite flocculation it may dissolve on standing or occasionally it may be definitely positive The blood test with this class is generally negative but may give a slight haziness and when early leishmaniasis infection is present in addition flocculation

may be seen with a hand lens We have lately devised a method of dilution by which doubtful sera can be differentiated from kala azar sera If the serum is diluted with eight to ten volumes of distilled water and then tested in the usual way no precipitate is observed as a rule with non kala azar serum but with undoubted kala azar serum a flocculent precipitate appears in the solution We have introduced the dilution method lately and have not fully studied its effects on the sensitiveness of the test in early cases of kala azar but so far as our observations go at present it does not appear to affect the reaction adversely (4) Fourthly totally negative sera in which no precipitate whatever is formed not even a slight haziness These are the sera from perfectly healthy persons and from those suffering from other diseases

Diagnostic value of the test

We have now performed this test on nearly 500 sera from both kala azar and non kala azar patients and have obtained corroborative evidence as to the diagnostic value of the test

In Table I we give a resume of the non kala azar sera on which this test has been performed A perusal of the table will show that out of 44 cases of acute malaria tested all gave negative tests Most of these cases had no enlargement of spleen or only slight enlargement and in all the diagnosis was made by finding malarial parasites in the blood The test was also entirely negative in filariasis dysentery sprue and epidemic dropsy Thirty cases of dermal leishmaniasis were examined 29 gave a negative reaction only one giving a strongly positive reaction and this was a case in which there was enlargement of the spleen Leishmania were found in the peripheral blood and visceral leishmaniasis was strongly suspected Of the 55 leprosy sera examined five were positive four doubtful and 45 negative the aldehyde test gave nearly corresponding results. It must be stated here that this series included ten cases of advanced leprosy which were specially selected because they give a positive aldehyde reaction Most of the positive sera came from these but whether these patients had leishmania infection also or whether in advanced leprosy changes similar to those occurring in kala azar are produced in the blood we cannot say as all these patients lived in the kala azar endemic area Of the six cases of leukæmia examined two gave a positive and four a negative reaction the same cases tested with the aldehyde test gave doubtful reactions in all six Of the nine cases of tuberculosis four severe cases gave a positive reaction to the antimony test and only four gave a doubtful reaction to the aldehyde test Of the 20 cases of skin disease 15 cases of syphilis a small fraction were doubtful but in the majority of them the test was entirely negative From the results of examination of 236 non kala azar sera analysed in the table one is therefore justified in concluding that the two reactions—antimony and aldehyde—run more or less parallel with each other and that their value in differentiating kala azar from non kala azar sera does not differ widely

TABLE I

Showing the comparative results of the antimony and aldehyde tests on non kala azar cases

No	Type of Case	Number of cases *done*	+++ and +	ANTIMONY TEST		ALDEHYDE REACTION			Confirmatory findings
				±	—	+++ and +	±	—	
1	Dermal Leishmaniasis	30	1	0	29	1	0	29	
2	Malaria	44	0	0	44	0	0	44	M P in all the cases
3	Filariasis	30	0	0	30	0	0	30	Four cases showed microfilaria in blood the rest with manifestations
4	Leprosy	55	5	4	46	7	6	42	Included ten advanced cases which were specially selected because they were said to give positive aldehyde reaction
5	Syphilis	15	0	2	13	0	0	15	W R positive in all cases
	Carried over	174	6	6	162	8	6	160	

TABLE I—*concld*

No	Type of Case	Number of cases done	Antimony Test +++ and +	Antimony Test ±	Antimony Test −	Aldehyde Reaction +++ and +	Aldehyde Reaction ±	Aldehyde Reaction	Confirmatory findings
	Brought forward	174	6	6	162	8	6	160	
6	Tuberculosis	9	4	0	5	0	4	5	Four cases which gave positive antimony test and doubtful aldehyde *reaction* were advanced (moribund) tuberculosis
7	Dysentery	8	0	0	8	0	0	8	Both amœbic and bacillary
8	Epidemic Dropsy	6	0	0	6	0	0	6	Clinical manifestation
9	Skin cases e g Dermatitis Leucoderma and Sarcoids etc	20	0	2	18	0	3	17	
10	Sprue	4	0	0	4	0	0	4	Clinical diagnosis
11	Leukæmia	6	2	0	4	0	6	0	By blood findings
12	Normal cases	18	0	0	18	0	0	18	
	TOTALS	245	12	8	225	8	19	218	

Advantages of the test

The antimony test has certain advantages over the aldehyde test Firstly the quantity of the serum required is smaller and with the blood test, even the process of removing blood from the vein (which is often alarming to the patient) and allowing time for its separation is not necessary Secondly the reaction is produced immediately and it is not necessary to wait for as long as 24 hours as is sometimes the case with the aldehyde reaction Thirdly we have deduced evidence to show that the antimony test manifests itself earlier than the aldehyde test

In Table II are given the results of examination of sera from kala azar patients tabulated according to the duration of illness It will be seen that the aldehyde reaction does not often develop for two or three months after the disease has set in the antimony test seems to appear much earlier In three patients, with duration of 10 to 12 days the test was doubtful of those in which the illness had lasted 15 to 21 days four gave a strongly positive and eight a positive reaction The aldehyde reaction was negative in all In those in which the disease had lasted for one to three months, out of 30 sera examined 17 were strongly positive and 13 were positive the aldehyde test gave doubtful or negative reactions in all In 237 cases of over three months duration whose sera were examined all gave positive antimony and aldehyde tests

Discussion

The antimony test when performed with a 4 per cent solution has been considered by some to be too sensitive as it gives a precipitate with a number of other sera especially those taken from cases of chronic malaria with enlargement of spleen Weaker solutions such as 1 0 per cent have therefore been suggested We have also noticed that the sera of some patients with enlargement of spleen give a positive antimony test but we have found it very difficult to exclude definitely leishmania infection in these in the endemic area in which we are working We have therefore undertaken to do a series of cases in the Punjab and have for that reason excluded from Table I doubtful cases of chronic malaria with enlargement of spleen which gave a positive antimony test We have tried solutions of different strengths ranging from 1 to 4 per cent and have found the last named to give more striking and distinctive results with most sera especially if 3 to 4 mm size test tubes are used The finger prick blood test also excludes many of the doubtful sera The purpose of dilution of the serum or of the antimony solution is here automatically served on account of the dilution of the blood which is used for the test and the disadvantages of the sensitiveness of the test are in this way eliminated

TABLE II.

Results of examination of kala-azar sera with the antimony and aldehyde tests tabulated according to the duration of illness.

No.	Duration	Number of cases done	Antimony Test				Aldehyde Reaction				Confirmatory findings.
			+++	+	±	−	+++	+	±	−	
1	10 to 12 days ..	3	0	0	3	0	0	0	0	3	Positive therapeutic test
2	15 to 21 days .	12	4	8	0	0	0	0	0	12	All 12 were diagnosed by finding of flagellates in the peripheral blood culture.
3	1 to 3 months .	30	17	13	0	0	0	0	4	26	In four cases peripheral blood culture was positive, in 18 spleen puncture was positive, and in eight there was marked leucopænia.
4	3 months onward ..	237	237	0	0	0	197	40	0	0	Cases from the Out patient Department, diagnosis made by *clinical and other findings*
	TOTALS ..	282	258	21	3	0	197	40	4	41	....

Summary and Conclusions

The antimony test is still in its infancy and much work must necessarily be done before its diagnostic value is properly established The principle however is established and the following are the conclusions we have been able to draw from our own experience with the test

(1) Observations on a large number of kala azar and non kala azar sera show that the antimony test is valuable in the diagnosis of kala azar While the test is more sensitive with the kala azar sera and gives more positive reactions, its value in eliminating non kala azar cases is shown to be nearly equal to that of the aldehyde test There is a likelihood of increasing it by the dilution method as our observations show that non kala azar sera never give a positive reaction in 1 in 10 dilution

(2) The advantages of this test over the aldehyde test are (*a*) A positive reaction appears earlier in the course of the disease than with the aldehyde test We have succeeded in getting a definitely positive test on the 15th to 21st day of the disease (*b*) The reaction is produced immediately and it is not necessary to wait for some hours as is sometimes the case with the aldehyde test (*c*) The test can be performed with much smaller quantity of serum

(3) The blood test described is much simpler requires little or no skill, and can be performed by the bed side

TABLE II.

Results of examination of kala-azar sera with the antimony and aldehyde tests tabulated according to the duration of illness.

No.	Duration	Number of cases done.	Antimony Test.				Aldehyde Reaction				Confirmatory findings
			+++	+	±	−	+++	+	±	−	
1	10 to 12 days	3	0	0	3	0	0	0	0	3	Positive therapeutic test
2	15 to 21 days	12	4	8	0	0	0	0	0	12	All 12 were diagnosed by finding of flagellates in the peripheral blood culture
								0	1	26	In four cases peripheral blood culture was positive, in 18 spleen puncture was positive, t there was marked

Anopheles and *Bironella* on the one hand and *Myzomyia* on the other With regard to the latter subgenus the species so far examined fall into three very distinct classes which we have called B D and F Only a single specimen of *A parangensis* one of a set sent by Rodenwaldt from Celebes has been examined The structure of the buccal cavity of this specimen resembled that of *A ludlowi* very closely and we have therefore included it in our class E Our class B which includes the group *Neomyzomyia* is remarkably distinct on the characters we have studied and would appear to warrant the separation of this group as a distinct subgenus as regards the form of the buccal cavity

The most important point on which we have based the grouping of species is in the form of the bucco pharyngeal armature in the female This in four of the groups is composed of a row of teeth arising at the posterior end of the buccal cavity and apparently forming a sieve between the cavity and the commencement of the lumen of the pharynx This armature is absent in the males In our class A this armature is also absent in the females This class includes the subgenera *Bironella* and *Anopheles* with the groups *Christya* and *Arribalzagia* of the latter subgenus

Class A The following species have been examined —

Name of species	Present classification: Subgenus	Group
A bironelli Chris	Bironella	
A a tken s James	Anopheles	Anopheles
A algeriens s Theo		
A barberi Coq		
A cul c form s Cog l		
A b furcatus L		
A pl mb us var *lar anens s* James		
A ma ul penn s M gen		
A as at cus Leic		
A annandalei Prashad		
A e seni Coq		
A l n les s C les		
A g gas var *s ml n* James		
A pun t pen s Say		
A ps u lopunc p n s Theo		
A cruc ans W l		
A hyr anus var *n gerrim s* C les		
A albotaeniatus Th		
A montanus Stanton and Hacke		
A s paratus Leic		
A umbros us Theo		
A novumbrosus Str cl l nd		
A barb rostris W lp		
A bancrofti C l s		
A grabham i Theo		
A implexus Theo		Christya
A vestit penn s D and K		Arribalzagia
A ps domacul pes Chagas		
A p c nacu la D and K		
A p nct n acula D and K		

In class B the bucco pharyngeal armature consists of a single row of large separate pectinate teeth 8 to 10 in number except in *A aureosquamiger* and *A watsoni* which have 12 to 14 This class includes the group *Neomyzomyia* of the subgenus *Myzomyia* and *A nili* formerly included in the group *Myzomyia* We find that *A christyi* which was provisionally placed in the group *Neomyzomyia* falls into our class E

We have examined the following species —

Name of species	Present classification	
	Subgenus	Group
A aureosquamiger Theo	Myzomyia	Neomyzomyia
A kingi Chris		
A watsoni Leic		
A kochi Donitz		
A leucosphyrus Donitz		
A tessellatus Theo		"
A punctulatus Donitz		"
A punctulatus var *moluccensis* Swellengrebel and S		
A annulipes Wlk		
A marstersi Skuse (*annulipes* Wlk)		
A amictus Edw		
A aurirostris Watson		
A nili Theo	"	Myzomyia
A umbrosus Theo of Edw 1911 (*nili* Theo)		

Class C The structure of the buccal cavity in this class is remarkably different from that of any other class It consists of two rows of teeth very markedly recurved so that in a dorsal view the ends are directed forwards, a feature seen in no other group examined The number of larger teeth is 8 to 12 and the posterior hard palate an area on the dorsal surface of the buccal cavity is very highly chitinized giving a cobblestone appearance *This class includes the* group *Nyssorhynchus* of the subgenus of the same name We have not been able to examine any specimens of the group *Kertes ia*

Four species have so far been examined —

Name of species	Present classification	
	Subgenus	Group
A argyritarsis R D	Nyssorhynchus	Nyssorhynchus
A albitarsis var *brasiliensis Chagas*		
A albimanus Wied		
A tarsimaculatus Goeldi		

Class D This class includes the majority of the *Myzomyia group of the* subgenus *Myzomyia* all the species of the group *Neocellia* which have so far been examined and one species of *Cellia* (*pulcherrimus*) The bucco pharyngeal armature consists of a double row of teeth without deep set roots and with about 12 to 14 teeth in each row, except in *A ramsayi* in which there are only 8 The line of origin of the teeth is only moderately curved

The following species have been examined —

Name of species	Present classification: Subgenus	Group
A rhodesiensis Theo	Myzomyia	Myzomyia
A culicifacies Giles		
A culicifacies var *adenensis* Chris		
A sergentii Theo		
A funestus Giles		
A subumbrosus Theo (*funestus* Giles)		
A funestus var *arabicus* Chris and Khazan Chand		
A listoni Liston		
A minimus Theo		
A aconitus Donitz		
A jeyporiensis James		
A jeyporiensis var *moghulensis* Chris		
A marshalli Theo		
A superpictus Grassi		
A transvaalensis Carter		
A rufipes Gough	,,	Neocellia
A stephensi Liston		
A fuliginosus Giles		
A philippinensis Ludlow		
A pallidus Theo		,,
A maculatus Theo		
A willmori James		
A theobaldi Giles		
A jamesii Theo		
A maculipalpis var *indiensis* Theo		
A karwari James		
A ramsayi Covell		
A pulcherrimus Theo		Cellia

Class E The bucco pharyngeal armature consists of a double row of teeth as in class D but in this case the teeth have long deep set narrow bases are usually more numerous (18 to 26 in each row except in *A ludlowi* and *A parangensis* which have 14 to 16) and the line of origin of the teeth forms a very pronounced curve This class includes the remainder of the group *Myzomyia* all the species of the Group *Pseudomyzomyia* so far examined two species of the group *Cellia* and *A christyi* which was referred to under class B *A pharoensis* and *A squamosus* which fall into this class are quite distinct from *A pulcherrimus* (Class D) in the structure of the bucco pharyngeal armature

The following twelve species have been examined —

Name of species	Present classification: Subgenus	Group
A subpictus Grassi	Myzomyia	Pseudomyzomyia
A vagus Donitz		
A ludlowi Theo	,,	,,
A ludlowi var *sundaica* Rodenwaldt	,,	,,
A parangensis Ludl		,,
A gambiae Giles (*costalis*)	—	--

In class B the bucco-pharyngeal armature consists of a single row of large separate pectinate teeth 8 to 10 in number, except in *A. aureosquamiger* and *A. watsoni* which have 12 to 14. This class includes the group *Neomyzomyia* of the subgenus *Myzomyia* and *A. nili* formerly included in the group *Myzomyia*. We find that *A. christyi*, which was provisionally placed in the group *Neomyzomyia*, falls into our class F.

We have examined the following species:—

Name of species	Present classification: Subgenus	Present classification: Group
A. aureosquamiger Theo.	Myzomyia	Neomyzomyia
A. kingi Christ.		
A. watsoni Leic.		
A. kochi Dontz.		
A. leucosphyrus Dontz.		
A. tessellatus Theo.		
A. punctulatus Dontz.		
A. punctulatus var. *moluccensis* Swellengrebel, 1918		
A. annulipes Wlk.		
A. marsteri Skuse (*annulipes* Wlk.)		
A. amictus Edw.		
A. aurirostris Watson		
A. nili Theo.		Myzomyia
A. umbrosus Theo. of Edw. 1911 (*nili* Theo.)		

Class C. The structure of the buccal cavity in this class is remarkably different from that of any other class. It consists of two rows of teeth very markedly recurved so that, in a dorsal view, the ends are directed forwards, a feature seen in no other group examined. The number of larger teeth is 8 to 12, and the "posterior hard palate," an area on the dorsal surface of the buccal cavity, is very highly chitinized giving a "cobblestone" appearance. This class includes the group *Nyssorhynchus* of the subgenus of the same name. We have not been able to examine any specimens of the group *Kerteszia*.

Four species have so far been examined:—

Name of species	Present classification: Subgenus	Present classification: Group
A. argyritarsis R.D.	Nyssorhynchus	Nyssorhynchus
A. albitarsis var. *braziliensis* Chagas		
A. albimanus Wied.		
A. tarsimaculatus Goeldi		

Class D. This class includes the majority of the *Myzomyia* group of the subgenus *Myzomyia*, all the species of the group *Neocellia* which have so far been examined, and one species of *Cellia* (*pulcherrimus*). The bucco-pharyngeal armature consists of a double row of teeth without deep-set roots and with about 12 to 14 teeth in each row, except in *A. ramsayi* in which there are only 8. The line of origin of the teeth is only moderately curved.

The following species have been examined —

Name of species	PRESENT CLASSIFICATION Subgenus	Group
	Myzomyia.	Myzomyia
A rhodesiensis Theo		„
A culicifacies Giles		
A culicifacies var *adenensis* Chris		
A sergenti Theo		
A funestus Giles		
A subumbrosus Theo (*funestus* Giles)		
A funestus var *arabicus* Chris and Khazan Chand		
A listoni Liston		
A minimus Theo		
A aconitus Donitz	„	
A jeyporiensis James		
A jeyporiensis var *moghulensis* Chris		
A marshalli Theo		
A superpictus Grassi		
A transvaalensis Carter		Neocellia
A rufipes Gough		
A stephensi Liston		
A fuliginosus Giles		
A philippinensis Ludlow		
A pallidus Theo		
A maculatus Theo		
A willmori James		
A theobaldi Giles		
A jamesi Theo	„	„
A maculipalpis var *indiensis* Theo		
A karwari James		
A ramsayi Covell		Cellia
A pulcherrimus Theo		

Class I The bucco pharyngeal armature consists of a double row of teeth as in class D but in this case the teeth have long deep set narrow bases, are usually more numerous (18 to 26 in each row except in *A ludlowii* and *A parangensis* which have 14 to 16) and the line of origin of the teeth forms a very pronounced curve. This class includes the remainder of the group *Myzomyia* all the species of the Group *Pseudomyzomyia* so far examined two species of the group *Cellia* and *A christyi* which was referred to under class B. *A pharoensis* and *A squamosus* which fall into this class are quite distinct from *A pulcherrimus* (Class D) in the structure of the bucco pharyngeal armature.

The following twelve species have been examined —

Name of species	PRESENT CLASSIFICATION Subgenus	Group
	Myzomyia	Pseudomyzomyia
A subpictus Grassi		„
A vagus Donitz		„
A ludlowii Theo		„
A ludlowii var *sundaicus* Rodenwaldt		„
A parangensis Ludlow	„	„
A gambiae Giles (*costalis*)		

In class B the bucco-pharyngeal armature consists of a single row of large separate pectinate teeth, 8 to 10 in number, except in *A. aureosquamiger* and *A. watsoni* which have 12 to 14. This class includes the group *Neomyzomyia* of the subgenus *Myzomyia* and *A. nili* formerly included in the group *Myzomyia*. We find that *A. christyi*, which was provisionally placed in the group *Neomyzomyia*, falls into our class D.

We have examined the following species :—

Name of species	PRESENT CLASSIFICATION	
	Subgenus	Group
A. aureosquamiger Theo.	Myzomyia	Neomyzomyia
A. kingi Christ.		
A. watsoni Leic.		
A. kochi Dönitz		
A. leucosphyrus Dönitz		
A. tessellatus Theo.	"	"
A. punctulatus Dönitz		
A. punctulatus var. *moluccensis* Swellengrebel and S.		
A. annulipes Wlk.		
A. mastersi Skuse (*annulipes* Wlk.)		
A. amictus Edw.		
A. aurirostris Watson		
A. nili Theo.		Myzomyia
A. umbrosus Theo. of Edw. 1911 (*nili* Theo.)		

Class C. The structure of the buccal cavity in this class is remarkably different from that of any other class. It consists of two rows of teeth very markedly recurved so that, in a dorsal view, the ends are directed forwards, a feature seen in no other group examined. The number of larger teeth is 8 to 12, and the 'posterior hard palate', an area on the dorsal surface of the buccal cavity, is very highly chitinized, giving a 'cobblestone' appearance. This class includes the group *Nyssorhynchus* of the subgenus of the same name. We have not been able to examine any specimens of the group *Kertes ia*.

Four species have so far been examined :—

Name of species	PRESENT CLASSIFICATION	
	Subgenus	Group
A. argyritarsis R.-D.	Nyssorhynchus	Nyssorhyncha
A. albitarsis var. *brasiliensis* Chagas		
A. albimanus Wied.		
A. tarsimaculatus Goeld.		

Class D. This class includes the majority of the *Myzomyia* group of the subgenus *Myzomyia*, all the species of the group *Neocellia* which have so far been examined, and one species of *Cellia* (*pulcherrima s*). The bucco-pharyngeal armature consists of a double row of teeth without deep-set roots and with about 12 to 14 teeth in each row, except in *A. ramsayi* in which there are only 8. The line of origin of the teeth is only moderately curved.

The following species have been examined —

Name of species	PRESENT CLASSIFICATION	
	Subgenus	Group
A. rhodesiensis Theo	Myzomyia	Myzomyia
A. culicifacies Giles		"
A. culicifacies var. *adenensis* Christ		
A. sergenti Theo		
A. funestus Giles		
A. subumbrosus Theo (*funestus* Giles)		
A. funestus var. *arabicus* Christ. and Khazan Chand		
A. listoni Liston		
A. minimus Theo		
A. aconitus Dönitz		
A. jeyporiensis James		
A. jeyporiensis var. *moghulensis* Christ		
A. marshalli Theo		
A. superpictus Grassi		
A. transvaalensis Carter		
A. rufipes Gough		Neocellia
A. stephensi Liston		
A. fuliginosus Giles		
A. philippinensis Ludlow		
A. pallidus Theo		
A. maculatus Theo		
A. willmori James		
A. theobaldi Giles		
A. jamesi Theo		
A. maculipalpis var. *indiensis* Theo		
A. karwari James		
A. ramsayi Covell		
A. pulcherrimus Theo		Cellia

Class I. The bucco-pharyngeal armature consists of a double row of teeth as in class D, but in this case the teeth have long deep-set narrow bases, are usually more numerous (18 to 26 in each row except in *A. ludlowi* and *A. parangensis* which have 14 to 16) and the line of origin of the teeth forms a very pronounced curve. This class includes the remainder of the group *Myzomyia*, all the species of the Group *Pseudomyzomyia* so far examined, two species of the group *Cellia* and *A. christyi* which was referred to under class B. *A. pharoensis* and *A. squamosus* which fall into this class are quite distinct from *A. pulcherrimus* (Class D) in the structure of the bucco-pharyngeal armature.

The following twelve species have been examined —

Name of species	PRESENT CLASSIFICATION	
	Subgenus	Group
A. subpictus Grassi	Myzomyia	Pseudomyzomyia
A. vagus Dönitz		
A. ludlowi Theo		
A. ludlowi var. *sundaicus* Rodenwaldt		"
A. parangensis Ludl		"
A. gambiae Giles (*costalis*)	"	"

Name of species	Present classification	
	Subgenus	Group
A. turkudi Liston	Myzomyia	Myzomyia
A. flaviceps Edw	"	"
A. multicolor Camb	"	"
A. cinereus Theo		"
A. christyi Newst and Carter	"	Neomyzomyia
A. pharoensis Theo	"	Cellia
A. squamosus Theo	"	"

Culicine Mosquitoes

We have examined 18 species of culicine mosquitoes belonging to 22 genera or subgenera and find that only those species included in the genera *Lutzia* and *Culex* (with the subgenera *Culiciomyia* and *Lophoceratomyia*) possess a bucco pharyngeal armature In the other genera in which the armature is absent, the structure of the buccal cavity is similar to that of the species falling within our class A of the Anophelines except in minor details

The teeth forming the armature in the buccal cavity of the females of *Lutzia*, of many species of *Culex* and of *Culiciomyia* are smaller and arise in a different position from those present in our classes B C, D and E of the Anophelines In some species of *Culex* and in the subgenus *Lophoceratomyia*, the teeth are, however, longer and sometimes very numerous, and number in some species more than 50 As in the Anophelines no teeth are present in the buccal cavity of the males

SOME ANOPHELES OF SARAWAK

BY

V A STOOKES, M C B SC , M B , D T M & H

I VENTURE to publish the following notes because there appear to be no recent data on the Anopheles of this country

In the thirteenth report on the Sarawak Museum, Moulton (1914) gives a list of the mosquitoes of Borneo which contains the names of twelve species of Anopheles as given below The four marked with an asterisk had not, at that time, been found in Sarawak

A brevipalpis, Roper *	*A ludlowi*, Theo *
A barbirostris, v d Wulf (sic)	*A maculatus*, Theo *
A umbrosus, Theo	*A leucosphyrus*, Don
A sinensis, Wied	*A punctulatus*, Don , var. *tesselatus*, Theo
A separatus Leic	
A albotæniatus Theo *	*A kochi*, Don

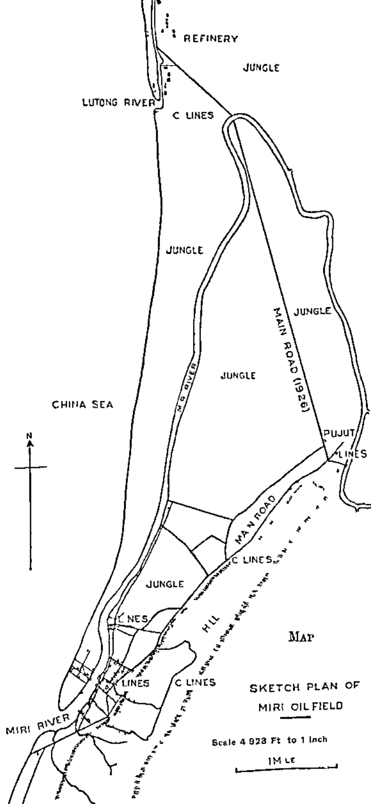
A karwari Theo

The only other publications I can find dealing with the Anopheline fauna of this coast of Borneo are those of Roper (1914) and Scharff (1927), with whose findings I compare my own Throughout this paper I also refer constantly to Swellengrebel's 'De Anophelinen van Nederlandsch Oost Indie (1921),' the only systematic description of Anopheles to which I have had access

Miri, on the Sarawak coast 600 miles from Singapore, in 4° 21' N Lat and 114° E Long , has in recent years become one of the important oilfields of the Far East, and the protection from malaria of those employed there has necessitated the observation and identification of the local Anophelines

The climate varies little, the average temperature ranging from 70°F to 98°F. and the relative humidity from 76 per cent to 100 per cent Heavy rains may take place at any time of the year though they are most common between October and February The average annual rainfall for the past five years is 117 inches

The oilfield stretches along the coast for 12 miles and is roughly triangular in shape, the apex of the triangle lying $4\frac{1}{2}$ miles inland The south eastern boundary is formed by a low range of hills, from 100 to 200 ft in height, the western face of which is scored by ravines containing streams, some of which are hidden by jungle


REFINERY
JUNGLE
LUTONG RIVER
C LINES
JUNGLE
JUNGLE
MAIN ROAD (1926)
JUNGLE
M R RIVER
CHINA SEA
N
PUJUT
LINES
MAN ROAD
C LINES
JUNGLE
HILL
MAP
LINES
LINES
C LINES
SKETCH PLAN OF
MIRI OIL FIELD
MIRI RIVER
Scale 4 923 Ft to 1 Inch
1 MILE

The soil between the hills and the sea consists of peaty loam sand and clay, it was until a few years ago, covered with jungle, with patches of mangrove swamp near the banks of the Miri River which runs from north to south through the length of the field Malaria chiefly of the subtertian variety, was at one time very prevalent, but has decreased greatly in amount, as the following figures show —

TABLE I

Showing the Malaria of Miri

	1922	1926
Labour Force .	3 300	3,200
Admissions to Hospital with Malaria	31 per cent	8 5 per cent
Out patient Attendances for Fever'	170	9 0

Anopheles brevipalpis Roper

Very few specimens of this mosquito have been found in Miri They agreed with the original description by Roper and their identity was kindly verified for me by Col S R Christophers About half a dozen adults all females were captured in isolated cooly lines near jungle at the end of 1923 and at the beginning of 1924 Since then no more have been seen probably because jungle has been cleared away from their breeding places No larvae were found at any time

Anopheles barbirostris v d Wulf

Specimens of this species were identified by Col Christophers Roper and Scharff both note its presence in considerable numbers Some years ago it was very plentiful in Miri and could be caught in most of the cooly lines during the day Now only two or three adults are caught in a month in spite of systematic searching presumably because the advance of civilization has destroyed many of the suitable breeding places

The larvae which are now very scarce were found in grassy pools and in overgrown stagnant drains usually in deep shade Other species found in the same places were *sinensis* (*hyrcanus*) or *separat is kochi* and occasionally, *ludlowi*

The table given below shows the recent decline in numbers caught and also the variation at different seasons

Three specimens were dissected in 1924 but although they were taken from the same lines as infected *ludlowi* neither stomachs nor salivary glands contained any parasites This small number of dissections is not sufficient to form the basis of an opinion but similar results were obtained in many more, of which I can now find no record and the impression I received was that this species played little or no part in spreading malaria

TABLE II

Showing numbers of barbirostris *caught in lines*

Months	1923 *	1924	1925	1926	1927
January		51	1	1	3
February		24	3	3	4
March		24	5	0	2
April		65	11	0	5
May		17	3	1	1
June		5	0	1	3
July		1	0	0	0
August		5	0	0	0
September		27	0	2	
October		61	1	0	
November		8	4	1	
December		1	0	0	

* No figures are available for 1923

Anopheles umbrosus, Theo

Specimens agreed in detail with Swellengrebel's description and with types sent me by Dr *Hacker* of the Malaria Bureau, Kuala Lumpur Roper found this species abundant in North Borneo and Scharff notes its presence in Labuan

Prior to 1924 it was common at the Refinery and was also found elsewhere in the oilfield Since the beginning of that year only two adults have been seen It was associated with *ludlowi* during the outbreak of malaria at the Refinery in 1922 and with *kochi* in a smaller outbreak at Pujut in July 1923, I have unfortunately no records of dissections done at these times and so no direct evidence of the part played by *umbrosus* I think, however, that it assisted in the former epidemic and probably originated the latter

Type 1 *Anopheles sinensis* Wied (*hyrcanus*, Pall)
Type 2 *Anopheles separatus*, Leic

I deal with these two species together because I am uncertain as to the identity of my specimens Roper found *separatus* in North Borneo, but not *sinensis*, Scharff recently took the larvæ of both in Labuan Swellengrebel (1921) maintains that *separatus* is merely a variety of '*M sinensis*,' although he states that his specimens do not agree exactly with those from the Malay States described by Strickland as '*M hunteri*' and later identified with *separatus* by Edwards Lamborn (1922) mentions the fact that *hyrcanus* is very variable in its characteristics, even the abdominal scale tuft sometimes being absent Walch (1923)

gives three photographs of the wings of '*M. sinensis*' ... *vanus*),' in only one of which a light mark on the fringe ca... the fifth longitudinal vein. The sole constant distinction ... seems to be the length of the apical fringe spot. This v... but in all of them is shorter than is generally described...

Four years ago I compared adults of what I thought ... from the Malaria Bureau and, to the best of my recollec... but, on looking through the recently obtained Anophel... paper, I found that all those labelled *sinensis* by the coll... rather than *hyrcanus*. There appeared, however, to be ... differed considerably from each other. The wing o... much smaller and gave the impression of being muc... other.

Differences in Wings of the Two ...

	Type I	
Costa	Small white spot at subcostal junction	Larg...
1st Longl. ..	Few white scales at distal end of inner ...	Larg...
	Few white scales below spot on costa	Lar... c...
2nd Longl.	Stem wholly dark	Lar...
4th Longl.	Stem wholly dark	Da...
Upper Br.	No white spot	Or...
Lower Br.	One white spot	Th...
6th Longl.	No white spot in distal half	La...

TABLE II

Showing numbers of barbirostris caught in lines

Months	1923*	1924	1925	1926	1927
January		51	1	1	3
February		24	3	3	4
March		24	5	0	2
April		65	11	0	5
May		17	3	1	1
June		5	0	1	3
July		1	0	0	0
August		5	0	0	0
September		27	0	2	
October		61	1	0	
November		8	4	1	
December		1	0	0	

* No figures are available for 1923

Anopheles umbrosus Theo

Specimens agreed in detail with Swellengrebel's description and with types sent me by Dr Hacker of the Malaria Bureau Kuala Lumpur Roper found this species abundant in North Borneo and Scharff notes its presence in Labuan

Prior to 1924 it was common at the Refinery and was also found elsewhere in the oilfield Since the beginning of that year only two adults have been seen It was associated with *ludlowi* during the outbreak of malaria at the Refinery in 1922 and with *kochi* in a smaller outbreak at Pujut in July 1923 I have unfortunately no records of dissections done at these times and so no direct evidence of the part played by *umbrosus* I think however that it assisted in the former epidemic and probably originated the latter

Type 1 *Anopheles sinensis* Wied (*hyrcanus* Pall)
Type 2 *Anopheles separatus* Leic

I deal with these two species together because I am uncertain as to the identity of my specimens Roper found *separatus* in North Borneo but not *sinensis* Scharff recently took the larvæ of both in Labuan Swellengrebel (1921) maintains that *separatus* is merely a variety of *M sinensis* although he states that his specimens do not agree exactly with those from the Malay States described by Strickland as *M hunteri* and later identified with *separatus* by Edwards Lamborn (1922) mentions the fact that *hyrcanus* is very variable in its characteristics, e.g. on the abdominal scale tuft sometimes being absent Walch (1923)

gives three photographs of the wings of '*M sinensis*' and '*M sinensis* (var *tanus*),' in only one of which a light mark on the fringe can be seen at the tip of the fifth longitudinal vein The sole constant distinction between the two species seems to be the length of the apical fringe spot This varies in my specimens, but in all of them is shorter than is generally described in *sinensis*

Four years ago I compared adults of what I thought were *sinensis* with types from the Malaria Bureau and to the best of my recollection, they were identical, but, on looking through the recently obtained Anopheles here, in preparing this paper, I found that all those labelled *sinensis* by the collectors resembled *separatus* rather than *hyrcanus* There appeared, however, to be two distinct types, which differed considerably from each other The wing of one (Type 1 below) was much smaller and gave the impression of being much darker than that of the other

Differences in Wings of the Two Types

	Type 1	Type 2
Costa	Small white spot at subcostal junction	Large white spot
1st Longl	Few white scales at distal end of inner ½	Large white spot
	Few white scales below spot on costa	Large spot twice length of that on costa
2nd Longl	Stem wholly dark	Light except basal spot
4th Longl	Stem wholly dark	Dark in basal half only
Upper Br	No white spot	One large white spot
Lower Br	One white spot	Three white spots
6th Longl	No white spot in distal half	Large white spot in distal half

The palps of Type 1 have a pure white tip those of Type 2 have a ring of dark scales round the lower half of the white tip giving it a dirty grey appearance The proboscis of Type 1 has a golden yellow tip at the base of which is a ring of pure white scales, in Type 2 the tip is much darker with a few yellow scales at its base Neither type has a scale tuft on the seventh abdominal segment

I am assured by the collectors that these have both been bred from larvæ of the *sinensis* type and that no larvæ without fans such as those of *separatus* have been seen. On examining the larvæ collected I found that they resembled those of *hyrcanus* in every respect except that the external clypeal hairs had not as many branches as would be expected and were more like those of *umbrosus*.

Five or six years ago these larvæ were very common in almost every shaded collection of water especially in the ravines where the streams were choked with fallen trees and debris and although adults were not frequently seen in houses and lines there was a considerable amount of malaria in all dwellings close to such breeding places. In consequence I formed the opinion as stated in a former paper (1923) that *sinensis* (as I then thought it was) must be a carrier. Certainly the abolition of the breeding places was followed by a reduction of the malaria, the percentage infected being now less than 0.5 per month.

I am unfortunately not in a position at the moment to consult any standard description of the two species or to compare my specimens with types, so that I give my findings as they stand and leave the question of determining the species till a more favourable time.

Anopheles albotaeniatus Theo.

Only eight specimens have been obtained, all of which were bred from larvæ in August 1927. They correspond in detail with Swellengrebel's description. Roper found only three specimens and Scharff did not record it in Labuan.

The larvæ were taken in a buffalo wallow, which also contained those of *leucosphyrus*, in an open grassy pool and in a stagnant drain, all of which were exposed to the sun.

There is no evidence that this mosquito carries malaria, but it has not hitherto been reported in Sarawak and thus is worth mention.

Anopheles ludlowi Theo.

This species, one of those noted by Moulton (1914) as unknown in Sarawak, occurs at times in very large numbers and is the most important malaria carrier here. The specimens agreed with Swellengrebel's description and with types from the Malaria Bureau, Kuala Lumpur. Roper found only a few individuals in North Borneo, but Scharff considers this to be the common carrier of malaria in Labuan. In the oilfield it was very much restricted in distribution, being confined almost entirely to the Refinery area although conditions seemed equally suitable elsewhere.

The larvæ bred in swampy pools just out of reach of ordinary high tides and in stagnant drains, wells, and even artificial containers, all of which were exposed to sunlight. As noted by Ter Poorten (1921), "When selecting its breeding places the chief attraction for *ludlowi* is the proximity of man, and in that case it may breed in dirty or in fresh water."

The very marked seasonal variation in numbers is shown in the following table with which I give a table showing the corresponding fluctuation in malaria.

TABLE III

Showing number of ludlowi *caught in the Refinery cooly lines*

Months	1924	1925	1926	1927
January	539	6	8	35
February	772	10	29	59
March	851	88	391	59
April	114	168	332	152
May	17	207	1 582	132
June	32	197	1 177	111
July	40	708	1 102	41*
August	124	429	121	
September	592	390	195	
October	829	415	157	
November	76	135	44	
December	5	7	28	

* July 1927 collection stopped on the 10th

TABLE IV

Showing percentage of Refinery coolies sent to hospital with malaria

Months	1924	1925	1926	1927
	Per cent	Per cent	Per cent	Per cent
January	4 5	1 3	0 8	0 6
February	5 5	1 3	2 2	0 8
March	7 8	1 1	1 8	0 6
April	9 3	0 9	1 0	0 2
May	2 2	2 8	1 7	0 5
June	3 0	3 3	6 9	1 1
July	2 2	1 8	3 1	1 4
August	1 3	8 2	2 1	0 7
September	4 2	8 4	0·9	
October	3 8	14 1	0 7	
November	6 3	4 6	0 3	
December	2 9	0 4	0 9	.

I have definite records of 22 dissections in January 1924 which yielded three infected stomachs a rate of 13 6 per cent This is sufficient evidence that *ludlowi* was an effective carrier The smaller numbers of *umbrosus* present in 1922 and 1923 may have assisted in spreading malaria but by 1924 they had completely disappeared

In order to reduce the malaria about £2 000 was spent between 1922 and 1925 in draining clearing and oiling near the Refinery but the numbers infected towards the end of the latter year showed that something more would have to be done to keep the disease in check Since the engorged *ludlowi* showed a marked inclination to remain all day in the nets of the coolies upon whom they had made their previous night's meal a more intensive campaign was instituted in 1926 to ensure their destruction, the tables show the greater numbers caught and the consequent reduction in malaria Le Prince (1926) has shown the value of this method of keeping down malaria in areas where other means of control are unsuitable or too costly, and James (1926) pronounces the dictum that The successful control of malaria lies not in the general knowledge that the disease is spread by mosquitoes of a certain kind, but in the particular and exact knowledge of the life history of a few individuals which succeed in becoming transmitters of the disease'

The reduction of malaria cannot, however, be at *of Anopheles alone* An attempt was also made to rer mosquitoes any human source of infection To this end complained of illness was examined for parasites and e in the area was examined at least once in six months, of illness or not All those found infected were remov the danger zone, where treatment was continued until at two successive weekly examinations Quinine was after the return from hospital and a final examination end of that time All new employees were examined at t went through the same routine

This procedure has of course, only been made pos co operation of the Refinery manager and his staff

I fully realize that such measures are only applica are under some sort of control, but both catching the pot and removal of the infectious malaria patient are mos combating a species such as *ludlowi*, with the breeding p to deal and whose house haunting habits render them ea

In considering the possible points of attack on th *variation in numbers gave rise to the thought that could t* be discovered it might be used as a means of control the presence of *ludlowi* on the banks of the Perak River amount of rain except near the mouth of the river The of the Lutong River and there appears to be no relation and the numbers of the mosquitoes found there Temp *shown by the table below* do not vary sufficiently to b

Swellengrebel quotes van Breemen's opinion that va water in breeding places controls the number of *ludlowi* *the highest concentration in which larvæ can survive as* higher than that of sea water and could only be arrived at I have not found so high a saline content necessary to larvæ, ordinary sea water kills the majority in a short ti consider the possibility that high spring tides might g numbers and I have prepared a chart showing the tides d a height of more than six feet and the numbers of *ludlowi*

It will be noticed that whenever the high tides follow *of less than ten days* the mosquitoes are very few in num the salinity of all pools reached by the tides is kept up aln

In the Dutch Indies several epiden ics of n alarıa are said to l ave been spread by *kocl ı* (Walcl 19?3 Bosch 19?5 Doorembos 19?5) but ıt does not seem to be of great ımportance as a carrıer here On one occasıon only l ave I found ıts ate l with that of malarıa ıt was together with *u brosı s* faırly l al n July 19?3 None of the specımens ı sponsible malarıa

and numbers of larva
cases are exceedingly rare

TABLE IX

Showing nu bers of kochı larvæ collected

Months	19?3	19?5	19?6	19
January	0	0	1?	9
February	0	30?	7	0
March	+	6?	0	0
Apıil	+	??	0	0
May	+	8	?6	43
June	+	8?	194	3
July	+	3 7	13?	41
August	+	?80	3?	11?
September	+	90	?0	
October	0	?17	0	
November	0	119	0	
December	+	19	0	

No larvæ were found ın 19 4

It ıs rather remarkable that neither *ı acı latus* nor *karwarı* l ave been foun here although Scharff commenting on the abseı ce of the former from Labua recalls that ıt ıs usually associated with a granıte formatıon It was found this coast by Roper and has I believe been responsible for much malarıa at Sata Island about 200 miles south of Mirı

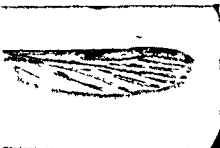
I wish to express my gratitude to Col S R Christophers and to Dr Hack for help ın ıdentifyıng Anopheles and also to acknowledge the debt I owe Mr Kerr Refinery manager and Capt Connor Marıne Superintendent at Mirı repeated assistance ın many ways

REFERENCES

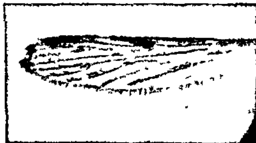
BOSCH W G (19?5) *Anopheles kochı* once aga n a Carrier of Ma *Geneesk T jd Ned Ind* Vol LXV No 6 pp 76?

PLATE VI

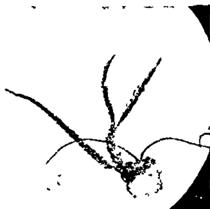
Type 1. *Anopheles sinensis.*
Type 2 *Anopheles separatus.*



Wing of Type 1



Wing of Type 2. (Same scale.)



Head of Type 1



Palps of both types

De Langen C D (1919) — Meded Burg Geneesk D enst in Ned Ind Anno 1919 Deel VI

Doorenbos W B (19 5) — Spec es San tat on an l D version to An mals as Measures to Control Malar a in the Trop s M D Thes Un of Leyden

Edwards F W (19 4) — A Synops s of the Adult Mosqu toes of the A stralas an Reg on B ll Ent o n Res Vol XIV No 3 pp 350—401

Hacker H P (19 0) — Malar a Bureau Reports Fed Malay States Vol II

James S P, an l Shute P C (19 6) — Report on the First Results of Laboratory Work on Malar a n En land League of Nat ons Health O gan zation Mal Com 19 6

Kotoltzky M (1925) — On the Spread and Prevalence of Malaria in Formosa Trans 6 h Cong F E A T M Tokyo 19 5 pp ? —31

Lamborn W A (19) — The Bionom cs of Some Malayan Anophel nes Bull Ent Res Vol XIII p 1 9

Le Prince J A (19 6) — De troy ng Engorged Anoph eles as a Malaria Control Mea ure U S Pul Heal h Rpts Vol LXI No 25 pp 1 20—1? 6

Moulton J C (1914) — Th rteenth Report on the Sarawak Museum 1914 S wk G vt Pr nt Off ce

Poper R (1914) — An Account of Some Anoj hel ne Mosqu toes found in N rt h N rtl Borneo w th description of a new spec es B ll Ent n R s Vol V No ? pp 137—14

Scharff J W (19 7) — Report on a Mosq to an l San tary S rvey of Labuan w th notes upon Ant mosqu to Measures proposed Ma yan Med Jour Vol II No 3 pp 88—93

Swellengrebel N H (19?1) — De Anoph el nen van Nederlandsch Oost-Indie No ? Druk 1971

Ter Poorten F H (19 4) — Some Remarks on M ludlowi and on the fight aga nst th s Mosqu to Rpts Dutch Ind an Med C v l S r ce 19 4 Vol I pp 99—114 Summ in B r App Ent om Se es B Vol XII p 9

Walch F W (19 3) — Some Remarks on Malar al Ep dem cs caused by M sens s (A hyrcanus) Trans 5th Cong F E A T M S ngapore 19 3 pp 46—71

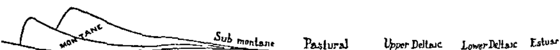
REGIONAL DISTRIBUTION OF ANOPHELINES AND MALARIA IN BENGAL

BY

M O T IYENGAR, B A, F Z S,
Entomologist Department of Public Health, Bengal

THE Province of Bengal is a compact piece of country extending from the Himalayas on the north to the Bay of Bengal on the south and enclosed on the east and west by smaller hill ranges It comprises areas with different physical features and as such it could be divided into definite regions such as the montane, the sub montane pastural upper deltaic, lower deltaic and the estuarine The montane region would include the mountainous area, the sub montane would be the foot hills The upper and lower deltaic regions are parts of the Gangetic delta above the reach of the tides and within tidal limits respectively The estuarine zone is close to the sea board A section through the province along a line running from north to south would pass through all these regions (Chart 1) It is possible to mark out these areas on account of their characteristic features

CHART 1



Section through the Province of Bengal from the Himalayas to the sea showing the different types of land formations

MONTANE ZONE

The Montane Zone covers the Himalayas on the north This zone consists of hills, ravines and cliffs rising abruptly with steep sides They are all clothed with dense forests of a tropical type at low elevations and of a temperate type higher up There is a heavy rainfall here the annual average being about 120 inches increasing considerably as we come to the foot hills The nature of the country is such that with the exception of stray rain pools and some sluggish streams there could be very few breeding places for Anophelines, the majority of the hill

streams usually rushing down with a torrential velocity The Anopheline fauna is accordingly poor only a few species being represented here *Anopheles gigas* and *lindesii* are found here above an elevation of 4 000 feet and breed in small rain pools *Anopheles aitkeni* breeds in forest streams that are not too rapid in their flow *Anopheles annandalei* is another hill species which breeds in tree holes All these three species are forest species *A willmori* is the fourth species found here in ravines which have been cleared of forest *A willmori* is the only species here known to transmit malaria the others are known to be perfect non carriers *Anopheles vagus* has been found to be fairly common in the hill villages

Sub Montane Zone

The Sub montane Zone consists of the foot hills The Darjeeling Terai the Bengal Duars and the foot hills of the Chittagong Hill Tracts come under this head This zone usually has a very heavy rainfall ranging from 180 to 2 0 inches of rain during the year mostly distributed between the months June to September The annual rainfall in the Chittagong Hill Tracts are very much less than in the Duars and Terai being usually about 100 inches of rainfall The land has a good slope and the soil is porous and consists chiefly of the detritus from the hills The area is traversed by numerous rivers streams and seepages of varying sizes and during the rains the number of such streams or jhoras as they are called is beyond apprehension On a single tea estate in a breadth of one mile as many as thirty five such streams come into existence during the rains These streams do not start flowing till the level of subsoil water in the hills above rises as a result of heavy rainfall there The local rainfall seems to have very little effect on them for it has been found that when the subsoil water of the hills is not high the streams do not hold water for more than a day even if there has been a heavy downpour locally Previous to the development of the tea industry the entire area was covered with dense tropical forests and heavy reed and grass savannahs often so thick as to be impenetrable by man These forests were cleared by a crude method known as juming a procedure still followed in some parts of Bengal for the purpose of bringing under cultivation land which is covered by dense forests The process of juming is as follows —In the months of January and February in the piece of forest land to be cleared all the smaller trees are felled and the branches of the larger trees are cut The cut branches are not removed but are allowed to dry in the sun till April when they are fired If there has been a thorough drying the firing reduces all but the large forest trees to ashes and burns the soil to a depth of an inch or two The ground is then cleared of charred logs and debris and the land is planted as soon as the rain sets in This was the procedure formerly adopted by the aboriginal tribes to clear these rain forests in the Duars and the Terai but the forests have since been cleared extensively to make way for the tea gardens, and at the present time the area covered by forests is very small

This region has a rich Anopheline fauna Fifteen species of Anopheles are common here of these the characteristic sub montane species are *A maculatus minimus, karwari jamesi jeyporensis theobaldi, kochi leucosphyrus* and *aitkeni* *A karwari* breeds in seepages *leucosphyrus* occurs in forest pools and *aitkeni* breeds in forest streams Some *willmori* occur in the higher regions of this zone while *culicifacies* is found in the lower sub montane areas, other species like *subpictus vagus fuliginosus barbirostris* and *sinensis,* which cover a wide range from the sub montane zone to the lower deltaic and beyond, are also present here

The sub montane region of Bengal is intensely malarious at the present day which could be attributed to the intensity of mosquito breeding during the wet season as also to the tropical aggregation of labour' on the tea estates here The effect of the clearing of the tropical forest of this region on the prevalence of Anopheles is nowhere brought out so characteristically as in the Bengal sub montane zone Four successive wet seasons I went into forests in the districts of Darjeeling and Jalpaiguri to investigate the species breeding in dense forests *In every one of these excursions* it was found that the forests here were very poor in Anophelines The species found within the forest streams were entirely different from the species found breeding in the continuation of the same streams in the area cleared of forest The streams within the forests bred *A barbirostris* and *aitkeni* and the forest pools bred *A leucosphyrus* As soon as the stream leaves the forest zone and is exposed to the sun *Anopheles maculatus* and *minimus* make their appearance in the cleared area and these species breed in very large numbers While the species which breed within the forests are non carriers those that breed in the cleared area are virulent carriers This shows the changes brought about by the clearing of forest on the Anopheline fauna in the sub montane zone Another fruitful source of Anopheles production in this area is the adaptation of the sides of the hills and the bed of streams for purposes of paddy cultivation The sides of hills are cut up into flat terraced plots In these either rain water is held in or a small hill stream is diverted into the top plot and water is thus continuously circulating in these terrace plots These terraces are prolific breeding places for *A maculatus* and *fuliginosus* It shows how a perfectly harmless piece of land can be turned out into a dangerous breeding place by the attempts of man to raise a wet crop in a region normally unsuitable for that crop It is also common to find the beds of streams embanked across along its entire course to hold in enough water to plant paddy seedlings

PASTURAL ZONE

The Pastural Zone is a dry region with a porous soil consisting largely of a ferruginous laterite gravel, sand and clay as in the area west of the Bhagirathi or composed of an old alluvium peculiar to this country locally known as the 'Barind The pastural zone extends more on the western portion of the

province than on the east The western and eastern portions of the pastural zone are two distinct formations The latter consists of an old alluvium the Barind' The western portion is mostly a laterite formation It is elevated and undulating and consists of a barren rocky and rolling country The country is traversed by numerous small streams which during the dry season are broad sandy beds with a tiny streak of water trickling down the centre but which soon after a heavy downpour of rain rise up very quickly and soon after subside to their former level There are no large trees anywhere and the vast stretches of land are covered with scrub jungle with dwarf trees and grassy wastes This zone stretches from the west to the east and includes a large part of the district of Midnapur Bankura and Birbhum and part of Burdwan Hooghly Murshidabad Dinajpur Bogra and of several other districts

This region has an interesting Anopheline fauna very different from that of the sub montane zone discussed previously *Anopheles culicifacies* is the most prevalent species of this region *A theobaldi listoni pallidus stephensi* and *maculipalpis* are the characteristic species of this region *A theobaldi* and *culicifacies* breed in the streams *A maculipalpis* breeds in seepages *A pallidus* and *fuliginosus* in rice fields and ponds Wells form the chief source of water supply in this area and as such large numbers of wells exist in this region These form suitable breeding places for *A stephensi* Another peculiarity of the wells of the laterite region is the extensive breeding in them of species like *A listoni varuna* and *culicifacies* which do not ordinarily breed in wells elsewhere

This region is recognized to be totally unfit for paddy cultivation *The Birbhum District Gazetteer* says Although the nature of the soil and the formation of land are unfavourable for the lodgment of water during the paddy season every effort is made to retain it in the fields and when it is stagnant it becomes the breeding ground of malaria bearing mosquitoes The people here have to resort to terrace cultivation if they desire to plant any paddy at all The sides of the hills especially in its lower levels are converted into tiers of rice fields often of the smallest size conceivable which are embanked along their lower edges The rain water in its downward course is arrested and instead of being allowed to pass down the hillside in a torrent is made to irrigate the fields one after another While it has been found that the rice fields within the deltaic regions are usually harmless as regards the breeding of Anophelines patchy terraced plots such as mentioned above are prolific breeding places of carrier Anophelines

In the pastural region there is usually very little malaria because of the porous soil and the facilities for a quick natural drainage But in some places malaria could easily be of the endemic type and these correspond with the zones in which there is much impounding of water for purposes of irrigation and water supply and much cultivation of terraced plots It has been found by Sir Leonard Rogers when he was working in the district of Dinajpur that those areas in which the level

of subsoil water is high are much more malarious than those areas in which the level of subsoil water is low, the converse of what happens in the deltaic regions lower down where, as we may see later, those regions with a low subsoil water are much more malarious than those with high subsoil water level

It happens in this type of country that man tries to adapt adverse conditions to suit his own purposes Streams are dammed to impound the water, sides of hills are converted for terraces for paddy cultivation Wells are sunk for water supply and when these are done without taking the proper safeguards against natural consequences, there is usually an increase of Anopheles breeding Another interesting feature of this tract is the subsidence of land as a result of extensive mining operations in the colliery areas with the result that the natural drainage of the country is interfered with and it is often found that as the result of such land subsidence the fall of rivers and their power to carry away water are interfered with All these factors serve to help the breeding of the Anophelines

Deltaic Bengal

The three zones discussed above, namely, the montane, the sub montane and the pastural are non deltaic, and are not covered by recent alluvium The three areas to be discussed subsequently, namely, upper deltaic, lower deltaic and estuarine, are deltaic in origin and composed of recent alluvium

Upper Deltaic Zone

The Upper Deltaic Zone —While the pastural zone is usually above the flood level, the deltaic areas are within the limit of the floods They are deltaic in origin and are alluvial The soil is composed entirely of recent alluvium and the annual rainfall is usually 60 inches in the western districts and 80 inches in the eastern districts It is traversed by a number of distributaries of the Ganges, some of which are alive while others are in a moribund condition This zone includes parts of several districts of Bengal, namely, Murshidabad Faridpur, Dacca, Mymensingh, Nadia, Burdwan, Hooghly Jessore and Khulna The following account given by Dr Thomas Oldham is a graphic description of the Bengal delta and its river system as a whole —

'The whole of the country lying between the Hooghly on the west and the Meghna on the east is only the delta caused by the deposition of the debris carried down by the rivers Ganges and Brahmaputra and their tributaries In such flats, the streams are constantly altering their courses, eating away on one bank and depositing on the other, until the channel in which they formerly flowed becomes choked up and the water is compelled to seek another course In this peculiar delta, the general course of the main waters of the Ganges has gradually tracked from the west towards the east, until of late years, the larger body of the water of the Ganges have united with those of the Brahmaputra and have together proceeded to the sea as the Meghna Every stream whether large or

small flowing through such a flat tends to raise its own bed or channel by the deposition of the silt and sand it holds suspended in its waters and by this gradual deposition the channel bed of the stream is raised above the actual level of the adjoining flats The consequence of this filling in and raising of its bed is that, at the first opportunity the stream necessarily abandons its original course and seeks a new channel in the lower ground adjoining until after successive changes it has gradually wandered over the whole flat and raised the entire surface to the same general level *

The Gangetic delta is traversed by the beds of the successive channels into which the Ganges distributed itself from time to time and which were formerly alive and which have since gone into decay as no water comes down through these channels since the heads have silted up In the comparatively unfilled in area in the eastern portion of the delta the rivers and distributaries are quite alive The upper deltaic zone could be divided into two regions (i) the western portion consisting of land which has been raised in this manner and is now traversed by a number of dead or partially dead distributaries and the land is generally *outside* flood influence and (ii) the eastern portion in which the land is still low and is subject to extensive flooding during the rains A description of the eastern region taken from the *District Gazetteer* shows the characteristic features of this part of the delta

'The district consists of low land inundated to a greater or less depth during the rainy season but yielding fine crops of rice and jute In the height of the inundation no land is to be seen and all travelling has to be done by boat To say that travelling has to be done by boat gives however but an inadequate idea of the real condition of affairs Half a dozen huts are clustered together on a little hillock a few yards square and the inhabitants cannot proceed beyond that hillock whether to visit their neighbours or their fields to go to market or to school without wading swimming or travelling in or on something that can float This expression is used advisedly for the people by no means confine themselves to boats For minor excursions rafts made of plantain trees are much in vogue or circular earthenware pipkins more difficult of navigation than a coracle A visitor to one of these hamlets in the rains may see a grey bearded patriarch swimming towards him from the fields and may be asked for alms by an old woman standing in water breast high amongst the jute plants

Every health report emphasizes the prosperity and non malariousness of the *eastern region* while the *western region* is notorious for its high malarial endemicity In the former the population is increasing the birth rates high and the death rates low while in the latter the statistics show that they are highly malarious The nearness of the subsoil water in this deltaic tract corresponds with non malariousness of the locality a contrast to what was found in the pastural zone

* *Proc Asiatic Society of Bengal* 18 0 p 47

The Anophelines of this region are *stephensi culicifacies pallidus philippinensis varuna pseudojamesi* and *theobaldi* There is a far greater prevalence of these species in the western delta than in the eastern portion *Anopheles stephensi breeds in the wells of which there is usually* a large number in the western portion of this zone *Anopheles culicifacies fuliginosus* and *philippinensis* breed profusely in the water courses and ponds *A varuna* and *pseudojamesi* breed in ponds In *addition to these there are the widely* distributed species *A subpictus vagus hyrcanus* and *barbirostris*

An interesting feature of the western portion of this region is that it is often subject to flooding when the rivers rise in floods during the monsoon season To prevent this there are numerous embankments alongside the banks of these rivers which embankments are often several strong one inside the other They exist in almost all the districts and even these strong embankments are broken through by these river floods and the entire country is covered with water thereby 'Such floods says the *Burdwan Gazetteer* ' are beneficial ' In 1909 the Banka overflowed its banks and flooded the surrounding country and a greater part of the town of Burdwan itself was under water for several days The vital statistics show that the flood serious and extensive as it was had no injurious effect on the health of the city and 1909 was one of the most healthy years Burdwan has ever experienced ' Such records are quite frequent in this area

Lower Deltaic Zone

The Lower Deltaic Zone comprises portions of the districts of Hooghly Bakarganj Noakhali Khulna Howrah and 24 Perganas The soil is entirely alluvial and the average rainfall varies between 60 and 85 inches during the year It was observed that the upper deltaic area is traversed by rivers and river beds in a moribund condition which were no longer getting their supply of water from the Ganges on account of the heads having silted up These rivers continue into the lower deltaic region herein discussed On the eastern portions of this zone the live rivers of the zone higher up also continue downwards towards the sea The connections of all these rivers with the estuaries are open and as such these rivers get the tides regularly These tides rush up and flood all surrounding low lying country which are connected with the tidal channels The country here is barely a few feet above the sea *level* and the depressions are mostly below the high tide level With the exception of the river banks the land is generally below the high tide level and it has been found that those areas as are subject to tidal influence are invariably *healthy* Those that are not reached by the tides are flooded over with storm water during the rainy season and they have invariably been found to be quite non malarious The banks of the rivers on the other hand are *elevated above the high tide level* and are usually endemic local areas The low lying areas with much stagnation of water are healthy The following description of the tidal region in the eastern

district of Bakarganj is taken from the *Gazetteer* "The country between the rivers is a maze of smaller rivers and petty streams to an extraordinary extent Most of the larger streams take their rise in the great rivers and most of the smaller streams join the larger streams together but every depression is surrounded by numerous watercourses and every homestead is surrounded by a moat which is usually connected with one of the smaller streams thus carrying on the work of distribution The country is also full of marshes not connected with the tidal rivers In this exceedingly water logged area there is no malaria whatsoever The western region is not as low lying as the eastern portion above described"

The Anophelines of this region are *minimus* var *varuna* *pseudojamesi* *philippinensis* and *tessellatus* *Anopheles culicifacies* is found in very few numbers The widely distributed species like *A subpictus* *vagus* *fuliginosus* *barbirostris* and *sinensis* are all common *subpictus* being particularly very common in this region The dry region species like *stephensi* and *pallidus* are absent here* and *culicifacies* is very rare *A vagus* is also sparse

In this area we have a very extensive network of tidal channels which flood the low lying country with water and the result has been a system of embanking all round to prevent the ingress of water and the result has been the exclusion of the tides from the land which was previously getting the benefit of the tides and as such there are instances of places having become more malarious as a result of these operations

Estuarine Zone

The Estuarine Zone is the last zone to be discussed It is on the sea board and comprises very low lying land which under natural condition is subject to flooding [illegible] The tides at the mouth of the rivers here rise very high [illegible] being as much as 12 to 18 feet [illegible]

a dense tidal forest [illegible]
tides rise very high the level of land being generally [illegible]
water level Mangroves predominate in this area and typical mangroves like *Kandelia* *Bruguiera* *Ceriops* and *Rhizophora* abound These islands are covered with dense forests composed chiefly of *Heritiera* The stemless *Nipa* and the dwarf *Phoenix paludosa* are characteristic palms of the swamps of this region Within the area subject to tidal flooding no Anophelines have been found to breed while within the embanked and protected area *A subpictus* *vagus*, *ludlowi*,

---

* *Anopheles stephensi* [illegible] has been observed [illegible] does not occur in the Lower Deltaic Zone but the City of Calcutta and the adjoining town of Howrah are exceptions where it has been found by the author that *A stephensi* breeds in large numbers in cisterns and tubs in this urban area.

fuliginosus sinensis and *barbirostris* breed commonly *A ludlowi* is a characteristic estuarine species not observed in other parts of Bengal

The littoral forests are known as the Sunderbans This region has been extensively cleared for purposes of paddy cultivation The process of clearing and bringing under cultivation this dense natural estuarine forest is a laborious task The dense growth of trees have to be cleared and the thick undergrowth is still more difficult to tackle It is not enough if the forests have been cleared for land to be planted over It is necessary to watch the cleared land as it would quickly relapse into its original condition if not carefully looked after If left to itself the land is rapidly afforested again or is invaded by a dense growth of reeds and the *entire area is converted into an extensive savannah more difficult to clear than* even the forests The next step is the prevention of the ingress of the saline tidal waters and this entails the construction of an embankment high enough to prevent the flooding of the land with water when the creeks rise in tides Lastly the soil which is impregnated with salt is in this condition unfit for planting and the salt has got to be washed out if anything by way of planting is to be done For this purpose several wash out sluices are put into the embankments these sluices which are made with wood are so constructed as to allow water from the land to flow into the river and to prevent the water of the river coming into the land The soil is thus washed out of salt slowly with every rainy season and the farmer has to wait for several years before he could think of planting the land with paddy When successive rains have washed out the salt of the soil to such degree as to enable planting to be made the cultivation starts

In these areas it has been found that on the land and side of the embank ments there are extensive breeding places of *A ludlowi* in the accumulations of brackish water which collect on the inside of the embankments and which cannot flow away Within the forests as also in the areas where the natural conditions have not been in any way modified there are no breeding grounds of this species Certain areas of the Sunderbans are notorious for their malariousness Even the paddy fields here breed *ludlowi* The entire area is so impregnated with salt that there are very few collections of water which are not brackish

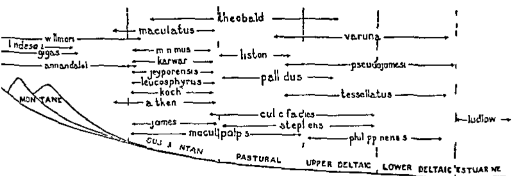
Range of Distribution of Species of Anopheles

I shall now proceed to summarize our present knowledge of the distribution of species of Anopheles in Bengal The material on which these observations are based are solely from Bengal and it is not known whether they hold good for other provinces of India The present information is based on work which has extended over ten years of frequent surveys all over Bengal and it is admitted that even much more information is needed to complete our knowledge of the distribution of the 28 *species so far recorded* from Bengal

The followmg is a list of the species of Anopheles so far recorded from Bengal —

1 1 (*Anopheles*) *a tken* James
2 *A* (*Anoph les*) *annandale* Ba ni Prasad
3 *A* (*Anopheles*) *barb rost is* Van der Wulp
4 *A* (*My.omy a*) *cul c fac es* G l s
5 *A* (*My.omj a*) *ful g nosus* G les
6 *A* (*Anoph les*) *g gas* var *s mlens s* James
7 *A* (*Anopl les*) *hyrcanus* var *n gerr mus* G les
8 *A* (*Myzomy a*) *james* Theobald
9 *A* (*My.omy a*) *jeypor ens s* James
10 *A* (*Myzomy a*) *karwari* James
11 *A* (*Myzomy a*) *kochi* Dön tz
12 *A* (*My om.yia*) *leucosphyrus* Don tz
13 *A* (*M.jzom.j a*) *l stoni* L ston
14 *A* (*Anopheles*) *l ndesa* Giles
15 *A* (*Myzomy*) *ludlo* Theobald
16 *A* (*Myzomy a*) *ma latus* Theobald
17 *A* (*My.omyia*) *mac l palp s* var *ind ens s* Theobald
18 *A* (*Myzom.yia*) *min mus* Theobald
19 *A* (*My.omy a*) *m n mus* var *varuna* Iyengar
20 *A* (*Myzomy a*) *pallidus* Theobald
21 *A* (*Myzomy a*) *ph l pp nen is* Ludlow
22 *A* (*Myzomy*) *pseudoj imes* Str kland and Chaudhur
23 *A* (*Myzomyia*) *stephensi* Liston
24 *A* (*Myzomyia*) *subp ct s* Grass
25 *A* (*Myzomy a*) *t ssellatus* Theobald
26 *A* (*My.omy a*) *theo ald* G les
27 *A* (*Myzom.yia*) *vagus* Don tz
28 *A* (*My.om j a*) *llmori* James

CHART 2



ct on thro gh the Province of Bengal showing the d fferent reg ons and the ran e of d tr but on of the spec es of *Anopheles* The w dely d str buted spec es of *Anopheles vagus sub p ctus ful g nosus barbirost is* and *hyrcanus* have been om tted from this chart

A provisional range chart of the distribution of the 28 species is given here (Chart 2) Five species are widely distributed *Anopheles subpictus* and *vagus* range from the montane to the estuarine but the former is more prevalent in the deltaic regions and the latter in the upper zones *A fuliginosus barbirostris* and *sinensis* also have a similar wide range from the sub-montane to the estuarine We shall now consider the less widely distributed species *Anopheles gigas* and *lindesaii* are confined to the montane zone *A annandalei* and *willmori* are montane but they frequently extend into the sub montane region *A minimus karwari leucosphyrus maculatus kochi* and *jeyporensis* are typically sub montane *Anopheles theobaldi* has a wide range from the sub montane to

the upper deltaic but occurs chiefly in the pastural zone *Anopheles culicifac* starts in the lower sub montane and extends through pastural and upper delt to a portion of the lower deltaic, but it occurs most profusely in the pastural zor *A listoni* and *maculipalpis* are confined to the pastural zone in Bengal *A pallid* is chiefly pastural in distribution but extends into the upper deltaic *A stephen* is pastural and upper deltaic in range *A philippinensis* and *pseudojamesi* a present in the upper and lower deltaic zones *A varuna* and *tessellatus* are al *of a similar distribution but they extend into the* pastural to a certain exter *Anopheles ludlowi* is confined to the estuarine zone

DISCUSSION

Major J A Sinton I M S (B India) I must congratulate Mr Iyengar on h very interesting and instructive paper This is a good example of the type of malar work which is being carried out in the provinces of India There appears to be a impression abroad that little or no anti malarial work is being done here This seen to be due to the fact that very few of the local reports ever receive a wide publicit Anti malarial work is being done in almost every province of India as an essential pa of the public health administration and malarial surveys have become so common th reports on them usually remain in the manuscript stage or, if published it is f local information only Widespread circulation of such reports is seldom considere necessary in any greater degree than other reports on local sanitary matters

A bibliography of the work on Indian malaria which includes references to a lar number of these reports is at present in the press It is suggested that Directo of Public Health should in future send copies of all locally published reports to th Tropical Diseases Bureau for review Such a step might do much to ensure that th extent of the anti malarial work which is being carried out in India was more widel known

Mr R Senior White (Bengal) Can Mr Iyengar mark on his admirable 'species map the approximate towns say on the Port Canning to Darjeeling route limiting c defining his sub regions of Bengal ?

Does water lying on colliery subsidences drain into abandoned workings ?

In his paper entitled 'A malarial survey of part of the lower Bengal delta' rea on Monday *A ludlowi* is not mentioned though the author was dealing with th region south east of Calcutta How near to Calcutta does *ludlowi* come ?

Is not true *rossi* mixed up with *ludlowi* in estuary regions ?

I should like to say that no other paper I have heard has interested me personally more

Sir Malcolm Watson (Federated Malay States) The photos of *ludlowi* breeding places and the dykes to keep out sea water remind one of the conditions in Malaya But the engineers of Malaya would not approve of the borrow pits being so broad o so close to the dykes The wide borrow pit is favourable to the breeding of the insect if the borrow pit is connected with a drain *ludlowi* will be more liable to be scoured out We have many thousands of acres of land in the 'ludlowi' zone free from malaria which formerly were very malarial

Lieut.-Col C L Dunn, I M S, (United Provinces) Anti malarial work in the provinces of India has, as Major Sinton says, been going on for years as part of the routine work of the public health department and hundreds of surveys have been carried out As the result of these surveys much money has been spent on anti larval work with in many areas, excellent results Very few of these have been published except for local circulation I was surprised to be told by Major Sinton when he spent a few days in Lucknow collecting many reprints from my office on those works, what the general impression outside a small circle of experts was and that the provinces of India were supposed to be doing little or nothing In the first report of the League of Nations conference there is a recommendation that anti larval work should be done in a few selected places in India This merely creates amusement in the minds of those who know what is being done but requires strong refutation in order to remove that world wide impression As one example of anti larval work I may say that the United Provinces Government, in connection with the building of the great Sarda Canal which has taken eight years to build and has cost eight million pounds have spent £30 000 on drainage and anti malarial work alone

Mr M O T Iyengar (Bengal) replied It is very difficult to draw a line across the map of Bengal and say that here this zone ends and the next one begins The different zones referred to by me in this paper, merge from one to the other so gradually that no sharp line of demarcation is possible between them The dividing line might easily be forty miles broad or just under two miles But judged on the whole, the zones are easily differentiated by the physical features of the country and the soil formations

Anopheles subpictus and *vagus* were not marked on the distribution chart as they have a very wide range of distribution from the estuarine to the montane zones *A subpictus* is very common in the estuarine zone and breeds alongside *A ludlowi* The former species is more prevalent in the lower zones and *A vagus* is sparsely so whereas in the higher regions like the sub montane and the montane, *A vagus* is very common and *A subpictus* is almost rare

A ludlowi is an estuarine species and is not present in the vicinity of Calcutta It is found only on the sea board It is common in the Sunderbans area 60 to 70 miles south of Calcutta, but can be found in smaller numbers about 30 to 40 miles south of Calcutta near Port Canning

the upper deltaic but occurs chiefly in the pastural zone *Anopheles culicifacies* starts in the lower sub montane and extends through pastural and upper deltaic to a portion of the lower deltaic but it occurs most profusely in the pastural zone *A listoni* and *maculipalpis* are confined to the pastural zone in Bengal *A pallidus* is chiefly pastural in distribution but extends into the upper deltaic *A stephensi* is pastural and upper deltaic in range *A philippinensis* and *pseudojamesi* are present in the upper and lower deltaic zones *A varuna* and *tessellatus* are also of a similar distribution but they extend into the pastural to a certain extent *Anopheles ludlowi* is confined to the estuarine zone

DISCUSSION

Major J A Sinton I M S (B India) I must congratulate Mr Iyengar on his very interesting and instructive paper This is a good example of the type of malaria work which is being carried out in the provinces of India There appears to be an impression abroad that little or no anti malarial work is being done here This seems to be due to the fact that very few of the local reports ever receive a wide publicity Anti malarial work is being done in almost every province of India as an essential part of the public health administration and malarial surveys have become so common that reports on them usually remain in the manuscript stage or, if published it is for local information only Widespread circulation of such reports is seldom considered necessary in any greater degree than other reports on local sanitary matters

A bibliography of the work on Indian malaria which includes references to a large number of these reports is at present in the press It is suggested that Directors of Public Health should in future send copies of all locally published reports to the Tropical Diseases Bureau for review Such a step might do much to ensure that the extent of the anti malarial work which is being carried out in India was more widely known

Mr R Senior White (Bengal) Can Mr Iyengar mark on his admirable species map the approximate towns say on the Port Canning to Darjeeling route limiting or defining his sub regions of Bengal ?

Does water lying on colliery subsidences drain into abandoned workings ?

In his paper entitled A malarial survey of part of the lower Bengal delta' read on Monday *A ludlowi* is not mentioned though the author was dealing with the region south east of Calcutta How near to Calcutta does *ludlowi* come ?

Is not true *rossi* mixed up with *ludlowi* in estuary regions ?

I should like to say that no other paper I have heard has interested me personally more

Sir Malcolm Watson (Federated Malay States) The photos of *ludlowi* breeding places and the dykes to keep out sea water remind one of the conditions in Malaya But the engineers of Malaya would not approve of the borrow pits being so broad or so close to the dykes The wide borrow pit is favourable to the breeding of the insect if the borrow pit is connected with a drain *ludlowi* will be more liable to be scoured out We have many thousands of acres of land in the 'ludlowi' zone free from malaria which formerly were very malarial

Lieut-Col C L Dunn, I M S, (United Provinces) Anti malarial work in the provinces of India has as Major Sinton says, been going on for years as part of the routine work of the public health department and hundreds of surveys have been carried out As the result of these surveys much money has been spent on anti larval work with, in many areas, excellent results Very few of these have been published except for local circulation I was surprised to be told by Major Sinton when he spent a few days in Lucknow collecting many reprints from my office on those works, what the general impression outside a small circle of experts was and that the provinces of India were supposed to be doing little or nothing In the first report of the League of Nations conference there is a recommendation that anti larval work should be done in a few selected places in India This merely creates amusement in the minds of those who know what is being done but requires strong refutation in order to remove that world wide impression As one example of anti larval work I may say that the United Provinces Government, in connection with the building of the great Sarda Canal which has taken eight years to build and has cost eight million pounds, have spent £30,000 on drainage and anti malarial work alone

Mr M O T Iyengar (Bengal) replied It is very difficult to draw a line across the map of Bengal and say that here this zone ends and the next one begins The different zones referred to by me in this paper merge from one to the other so gradually that no sharp line of demarcation is possible between them The dividing line might easily be forty miles broad or just under two miles But judged on the whole, the zones are easily differentiated by the physical features of the country and the soil formations

Anopheles subpictus and *vagus* were not marked on the distribution chart as they have a very wide range of distribution from the estuarine to the montane zones *A subpictus* is very common in the estuarine zone and breeds alongside *A ludlowi* The former species is more prevalent in the lower zones and *A vagus* is sparsely so whereas in the higher regions like the sub montane and the montane, *A vagus* is very common and *A subpictus* is almost rare

A ludlowi is an estuarine species and is not present in the vicinity of Calcutta It is found only on the sea board It is common in the Sunderbans area 60 to 70 miles south of Calcutta but can be found in smaller numbers about 30 to 40 miles south of Calcutta near Port Canning

PARASITIC NEMATODES OF *ANOPHELES* IN BENGAL

BY

M O T IYENGAR, B A, F Z S,

Entomologist, Department of Public Health, Bengal

DURING the course of routine identification of larvæ of Anopheles from one of the larvæ that were then under the microscope, a long white worm suddenly emerged out into the water and began to move about on the slide This observation was made on a larva of *Anopheles minimus* var *varuna* Iyengar and it has since been observed in larvæ of other species of Anopheles as well These worms belong to the Genus *Mermis* family *Mermithidæ*, the specific determination has not yet been made These worms have been studied in some detail their life history and the interesting method of infection have been worked out and are described in the present paper

In the earlier stages of the parasite it is difficult to find out whether a larva is infested with the worm or not, but when the worm is fully developed it can be seen coiled up within the parasitized larva The parasitized larva usually has a slightly thicker body than the normal larva and in the region of the thorax we can notice the loops of the worm The worm lies coiled up within the body cavity of the larva from the thorax to the fifth or sixth segment of the abdomen and running as many as three or four lengths within the body It lies on both sides of the alimentary canal and ventral to it between the alimentary canal and the ventral nerve cord and the ventral body wall When the worm is mature it exhibits a slow creeping motion within the body of the host When the worm is ready to emerge out of the body of the host its movements are much quicker, its loops get stronger, stiffer and more obtuse thereby swelling up the body of the larva and thus causing it to rupture The worm then emerges out through the tear thus occasioned and thereafter lives a free life In all the instances so far observed only one worm emerges out of a single larva When the worm has emerged out the body wall of the host collapses altogether as the rupture caused by the worm causes the loss of the body fluid and the host is killed instantaneously All that is left of the host after the worm has emerged out is the collapsed body of the larva Usually the head of the worm emerges out through the rupture first and the worm then slides out very quickly into the water the whole process taking little more than ten seconds Occasionally, however the tail is the first to come out, in which case the emergence of the worm is much delayed

Figs 1 and 2 (Plate VII) are photomicrographs of *Mermis* killed while partially emerged from the body of the host In these pictures a consider able part of the worm is still within the body of the Anopheles larva Fig 3 shows the worm lying within the body of a larva of *Anopheles philippinensis* one day prior to its emergence from the host Fig 4 is a more magnified photograph of the thorax of the larva showing the head (on the left) and the coils of the worm

Serial sections of parasitized larvæ show that the worm lives within the body cavity They show that the worm has ample space to live and move about within the body cavity as most of the abdominal muscles have not developed and there is very little of fat tissue within the body of the parasitized larva (Plate VII fig 5) It is therefore easy for the worm to move about within the body cavity from the thorax to the sixth abdominal segment Larvæ infested by these worms do not appear to suffer much inconvenience as they pass through the moults quite normally and reach the last larval instar as do healthy larvæ But they have not been observed to pupate Serial sections show that the para sitized larva is lacking as regards imaginal and pupal parts as the wings and legs of the future insect are either totally wanting or are present as mere rudiments The presence of the parasite evidently suppresses the development of the imaginal organs It has invariably been found that the worm emerges out when the host is still in the larval stage

The present observations have mostly been conducted in rural areas of the Lower Bengal Delta and it has been found that the parasitization of Anopheles larvæ is especially common during the monsoons in the months of August and September Seven species of *Anopheles* have so far been found to be prone to attack by these worms They are *Anopheles varuna pseudojamesi sinensis barbirostris fuliginosus philippinensis* and *tessellatus* It is likely that larvæ of other species are similarly parasitized by these worms

I have not been able to get at any previous records of these worms from India with the solitary exception of a mention by Ross (1906) of a nematode found in a Culex larva He says — Three large active but immature nematodes were found in the stomach cavity of a single larva of a Culex (probably *C fatigans*) obtained near Ootacamund India I never saw these parasites again It is possible that they belonged to the Genus *Agamomermis* Styles With the exception of this solitary reference of worms from Culex larva there has been practically no information regarding worms infesting mosquitoes in India

The worm when it emerges from the larva of *Anopheles* is long white and opaque It ranges from 5 mm to 8 mm long and is usually from 63 μ to 110 μ thick The thinner and shorter worms are the worms which become males later on while the thicker and longer ones are the future females The anterior end is somewhat pointed and has a thin vestigial alimentary canal The posterior end is pointed and ends in a papilla At the time of emergence from the host these worms are still in the embryo stage and are sexually immature

Besides worms which have actually emerged out of Anopheles larvæ numerous Anopheles larvæ were dissected for parasitic *Mermis* The worms that were thus dissected out of the host larvæ were very immature and varied considerably in size They were from 2 8 mm to 7 0 mm long and from 27 μ to 75 μ thick according to the condition of development of the worm These immature stages have a slightly thicker head end and the tail end is uniformly pointed and not suddenly constricting out into a papilla as in the more developed form *Mermis* when it emerges out of the host is not sexually mature the completion of its sexual development occurs outside the body of the host in a free living state After emerging out of the host it does not feed any longer and it is therefore furnished with a good store of fat within its body on which it lives its subsequent free living non feeding life

Various methods were tried to keep these embryo worms alive in order to let them complete their sexual development but none was found to be as efficacious as a dish with a wet sand slope and with a little water at the base of the dish It was found that the worms flourished very well in these wet sand slopes and that they could be kept alive in this manner for over two months No special attention was necessary for keeping them alive beyond occasionally adding a little water to make up for the evaporated water and to keep the sand continually wet Within three weeks these worms became sexually mature The male worms were generally thinner and measured 4 4 mm long and 55 μ to 63 μ thick The females measured 11 mm long and 110 μ to 150 μ thick The head end was thinner and measured 80 μ in thickness By this time at least one moulting had taken place and the papilla at the posterior end found in the worm when it emerged from the *Anopheles* larva has disappeared the posterior end of the sexually mature worm is rounded By this time all the reserve fat of the embryo has disappeared and the worm is much thinner than the original embryo

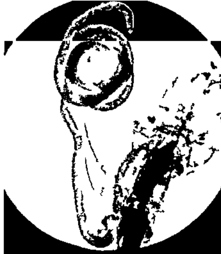
Male and female worms were kept together in moist sand slopes and the water in the dish was being examined frequently Nothing noteworthy happened until one day a tiny microscopic worm was found wriggling about in the water This was the young one of the worm and when a small quantity of the water in the tray was treated with 10 per cent formalin and centrifuged similar worms were found in large numbers in the precipitate They measure 1 mm to 1 2 mm long and 12 μ to 14 μ thick but are usually 1 1 mm by 13 μ The anterior end is blunt and its posterior end tapers into a point and the tip is curved (Plate VII fig 6) In spite of frequent examination no eggs of these worms were found until the worm larva was encountered and I am led to believe that these worms are probably viviparous unless it be that I have missed the eggs Further work will clear this point

The method of infection of the Anopheles larvæ has also been studied in some detail The young worms are extremely active and move rapidly along the water surface They rarely sink below the surface of the water This habit of these worm embryos to move along the surface of the water gives them the maximum chances of attacking Anopheles larvæ which also float along the

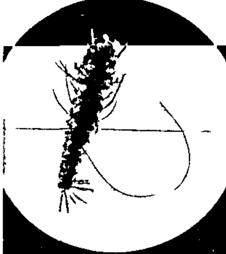
surface of the water. The method of infection of the larvæ by the young worms is by penetration of the body The moving worm on coming in contact with the Anopheles larva, holds on to it quickly by curling round a lateral branched hair of the larva and later by gripping it by means of its prehensile tail Having secured a hold of the larva it curls round the hair or the thickened base of the hair and attacks the body wall With its anterior end it tries to dig into the body of the Anopheles larva having gained a purchase in the twists which it has made over the hair and the base of the hair While trying to dig into the body of the larva the worm embryo makes a forward and backward movement in the fashion of a gimlet the anterior end being maintained perpendicular to the body wall The worm embryo appears to be provided with a fine transparent partially retractile boring spicule which enables it to effect an entry into the tissues of the host Having inserted its anterior end into the body it curls round within the body of the host, releases its hold on the outside and draws itself into the host The whole process from the time of attachment to the time of complete entry takes from one to two minutes sometimes slightly longer Having entered the host the *Mermis* embryo moves about a great deal within the body cavity and can be seen moving rapidly from place to place in the head, thorax or abdomen A day after its entry into the host its movements are very feeble and it is then difficult to recognize it owing to its great transparency It grows rapidly, and after a few days it is generally to be found ventral to the alimentary canal between the fifth and second abdominal segments The embryo grows at a remarkable rate and at the end of eight to ten days after the time of entry it has grown from an original size of 1 mm by 13 μ to a size of 8 mm by 75 μ to 160 μ The mature embryo then emerges out of the Anopheles larva before the latter pupates No moulting of the embryo has been observed to take place within the host larva The embryo has no alimentary canal and it imbibes nutriment through its thin skin from the cœlomic fluid in which it lives

Under laboratory infection experiments, as many as twenty *Mermis* embryos effected their entry into a single Anopheles larva in the course of one night of exposure to infection But in nature only one embryo is to be found within a single Anopheles larva as shown by the numerous dissections of Anopheles larvæ collected from natural breeding places Even in laboratory infection experiments although in the freshly infected larvæ, the number of embryos was many, the number gradually got less day by day and after four days only one embryo was found in each larva The fate of the embryos that entered the larva is not definitely known

Another remarkable observation is that in some ponds Anopheles larvæ were found parasitized to a large extent while in others, the larvæ were free It was always easy to get infected larvæ by collecting them from these ponds It is proposed to experiment on the possibility of infecting other ponds with these worms or their embryos to see if such an introduction would stay These observations seem to open out a wide field for further work



F 1



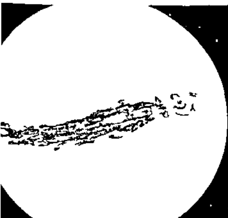
Fig

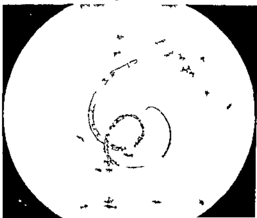


F 3



Fig 4





It was mentioned previously that seven species are liable to parasitization by worms of the Genus *Mermis* Anopheles the specific determination of these worms has not yet been made The specimens show some variation in size and it is likely that these worms belong to more species than one

Mermis Infesting Adult Anopheles

So far, we have been discussing the *Mermis* which parasitize larvæ of Anopheles None of these have been found to pass on to the adult mosquito since invariably the worm emerges out before the larva pupates In Bengal, certain species of *Mermis* also parasitize adult Anopheles mosquitoes These are different from the ones previously discussed as they are much bigger in size They measure from 14 mm to 17 mm long and 168 μ to 174 μ thick A few smaller forms have also been found measuring 8 3 mm × 138 μ It is not sure if the latter smaller worms are only the males or they are of a different species As with the species of *Mermis* which infest the Anopheles larva these worms which infest adult *Anopheles* are sexually immature at the time of emergence from the host As the further development of the worm is in water or in wet earth the worm emerges out of the mosquito only when it is close to water The mosquito is killed as soon as the worm emerges out and the worm enters into the water It is surprising to see how a worm over 17 mm long and nearly a fifth of a millimetre thick lies coiled up within the body of an adult mosquito Two species of Anopheles have been found thus infested namely *A fuliginosus* and *A subpictus* The further development of the worm and the manner of infection has not been studied

I am indebted to Dr H A Baylis of the British Museum for determining the genus of the worm from the Anopheles larva

Reference

Ross P (1906) Notes on the parasites of mosquitos found in India between 1895 and 1899 *Jour Hyg* Vol VI pp 101—108

Discussion

Bt Col S R Christophers I M S (B India) (Chairman) Most of us who have dissected mosquitoes will be familiar with some form or other of the nematode parasites of adult and larval mosquito states described by Mr Iyengar and it is therefore a great pleasure for me as I think for others here to have listened to Mr Iyengar s very complete investigations All parasites of the mosquito have a possible importance since they are natural enemies and may thus possibly come into the picture of disease occurrence or prevention I hope some here may have some further observations to give on these forms

Major J A Sinton I M S (B India) The presence of nematode larvæ in any stage of the mosquito has always been of interest to workers in tropical medicine since Manson s classical discovery of the transmission of filaria by the mosquito

From the common occurrence of microfilaria in animals, especially birds, in India the presence of nematode larvæ in adult mosquitoes is not to be wondered at, but their *presence in the larval stage* is apparently much rarer

About 25 years ago Stiles described a nematode parasite, *Agamomermis culicis* in mosquito larva in America He found that this parasite either killed the larva as mentioned by Mr Iyengar, or if the larvæ survived and developed into adults, the ovaries were undeveloped and egg laying was hindered It was suggested at one time that this parasite might be used for the destruction of mosquitoes, but I believe that the results of the experiments were not encouraging

Perry while working in the Jeypore Hill Tracts in India, recorded a nematode larva in Anopheles which he believed inhibited egg production He thought the infection was acquired in the larval stage Similarly Carter in Ceylon has recorded nematode larvæ from mosquitoes in both the pupal and larval stages A similar parasite has been mentioned in the yellow fever mosquito in Africa

Personally I have no experience of nematode infestations of mosquitoes, but during a malaria enquiry at Kohat N W F Province, the occurrence of an encysted trematode larva (*Agamodistomum* sp) was not uncommon in both adult and larval Anopheles Some of the insects were very heavily parasitized, as many as 250 cysts being found in a single specimen Soparkar in Bombay found other stages of this parasite in fish and snails

Mr R Senior White (Bengal) My assistant on the Bengal Nagpur Railway, Adhikari found a nematode in a *barbirostris* larva I would like to know in what stage Anopheline larvæ are attacked and what percentage of parasitization is found in nature ?

Major H E Shortt, I M S (B India) Although it has no direct connection with the subject discussed by Mr Iyengar, I think it may be of some interest to record the experience of the Kala azar Commission with nematode parasites of *Phlebotomus argentipes* The latter insect was being artificially reared in large numbers and owing to the confined space in which this was done it was found that a large percentage became infected with a parasitic nematode which occurs although comparatively rarely, in flies caught in nature

The adult form of the worm was commonly found in the larvæ of *P argentipes* and the eggs and larval forms in the adult of that insect The infection in time became so intense that the young worm larvæ which found access to the ovarian tract of the fly, prevented oviposition by the latter and so led to its death Practically 100 per cent of the flies became infected and were thereby rendered unsuitable for experimental work with *Leishmania* necessitating the rejection of the entire stock of flies and a fresh commencement of breeding with clean flies

Mr M O T Iyengar (Bengal) In reply to Mr Senior White, I may say that three genera of *Mermithidæ*, namely, *Agamomermis*, *Limnomermis* and *Paramermis*, have been recorded from mosquito larvæ These records have all been from Culicines, with the solitary exception of a record of *Limnomermis* from *Anopheles* sp There has been no record of these parasitic worms from India with the exception of the mention by Ross (1906) in which three worms were found in a Culex larva Styles' paper is a splendid contribution on the habits of *Agamomermis* In the present paper, the

life history and method of infestation has been studied in some detail In regard to the percentage of infection the infestation seems to be fairly common during the rains but there is considerable variation While in some ponds a good percentage of the Anopheles larvæ are found parasitized in others there were very few of them parasitized

The stage of the development of the larva at which infection normally happens is the first instar or the early part of the second instar The older grown up larvæ have not been found to be attacked by the young larval *Mermis* although that might happen in laboratory infection experiments

The different moults of the larva occur without any hinderance I have not noticed any difference in the duration of the different instars although that seems but reasonable between the parasitized larva and the healthy ones Regarding the query whether, in case the worm does not emerge from the larva the larva pupates or dies, I may say that as it has invariably been found that the worm comes out of the parasitized larva when still in the larval stage no such observation has been possible The larva has not been found to pupate The *Mermis* found in the adult Anopheles is different from the ones found in the larva The larvæ infested by *Mermis* have not been observed to pass on to the adult mosquito

MICROSPORIDIAN PARASITES OF ANOPHELES LARVÆ

BY

M O T IYENGAR, B A, F Z S,

Entomologist, Department of Public Health Bengal

ANOPHELES larvæ in lower Bengal are subject to parasitization by *Microsporidia* of the Genus *Thelohania* The infection by these micro organisms is often so common and so heavy that in some seasons of the year there is considerable mortality among Anopheline larvæ attributable to these parasites Six species of Anopheles have so far been found to be subject to such infection, namely, *A varuna*, *pseudojamesi*, *fuliginosus*, *barbirostris*, *hyrcanus* and *subpictus*, and it is likely that other species of Anopheles in Bengal are liable to a similar affection About three species of *Thelohania* seem to be concerned with this infection but they have not been determined yet

Feebly infected Anopheles larvæ exhibit a translucent opacity in the affected region In a more advanced stage, the infected segments of the body show a dense whitish opacity in marked contrast to the transparency of the unaffected segments (Plate VIII fig 1) At a still later stage, the affected segments are distinctly swollen Usually it is the thorax and the first one or two segments of the abdomen that are affected and from the thorax the affection gradually proceeds posteriorly But it also happens that the abdominal segments are infected while the thorax remains unaffected Sometimes I have noted the occurrence of two foci of infection in a single larva one in the thorax and another in the 8th and 9th abdominal segments, the intervening region remaining healthy

Other than the opacity and bloated appearance of infected larvæ, they do not differ materially in their habits from healthy larvæ, as they go through the larval moults as normally They feed and move about as healthy larvæ do It is only when the disease has much advanced, and the body segments are considerably swollen up that the host loses all power of feeding and motion, and at this stage the only signs of life in the larva is the contraction of the heart seen under the microscope Soon after this stage is reached, the larva dies

The infected segments of the host body are packed up with an enormous number of microsporidian spores On crushing the larva and examining the fluid under the microscope innumerable numbers of minute highly refractile oval spores are to be seen in the fluid, either scattered singly or in groups of eight spores enclosed in a transparent envelope The spores have a vacuole at one end, and they exhibit

brownian movement The refractile nature of the spores and the presence of the vacuole are characteristics of a microsporidian spore, but the confirmation rests in the extrusion of the polar filament from the spore After trying various chemicals for the extrusion of the polar filament I found that satisfactory extrusion was to be had only by mechanical pressure Kudo (1924 p 58) suggests the following simple procedure for the extrusion of the polar flagellum by mechanical pressure 'A very small drop of water emulsion of fresh microsporidian spores is placed upon a slide Place the slide on a smooth and steady surface and cover the coverglass with a piece of cloth or filter paper over which the elbow is gently applied Give a strong downward push to the arm This will instantly cause the extrusion of the polar filament By this method the long polar filament could be extruded from the spores and could be seen by dark ground illumination *Many of the flagellæ were attached to the spores while a few were found* detached The basal portion of the flagellum is very much thickened while the apical portion is quite thin These flagellæ can be seen in Plate VIII fig 6

Smears of water emulsion of the parasitized larva were fixed and stained and showed the spores the octosporous sporonts and the immature forms On account of the impermeability of the spore membrane it is usually difficult to stain these spores when stained with any of the Romanowsky stains the spore shell takes the eosin but the internal structure is not clearly seen The best method of staining these spores is to fix them in Bouin s fluid (picro formol acetic) wash in water and stain with iron hæmatoxylin I have had splendid results by over staining with strong Delafield s hæmatoxylin reducing with an alcoholic solution of picric acid and counter staining with eosin (Plate VIII fig 5) By this process the spores are stained excellently But this procedure was found less satisfactory for the vegetative forms for which I found Giemsa solution useful in bringing out the finer details of the cells

Serial sections were cut of parasitized larvæ and it was found that the parasites were entirely confined to the fat body of the host Within the region of heavy infection the fat cells had been entirely invaded and in their place were sacs containing the spores of *Thelohania* (Plate VIII fig 3) In the regions not yet fully infected where the fat body was being gradually invaded some portion of the fat body was healthy while others were already entirely eaten up A photograph of a section of this region shows a portion of the fat body partially invaded by the microsporidian and shows the difference between healthy fat cells and the invaded fat cells (Plate VIII fig 4) It was mentioned that the invasion of the fat cells extends gradually from the original site of infection in the thorax towards the posterior segments A longitudinal section of the infected larva shows the progress of the invasion In the segments nearer to the primary infection the parasites consist almost entirely of spores and sporonts with vegetative forms in a very small proportion while in the region at some distance from the site of original infection the fat cells are occupied by parasites in which the vegetative forms are more common

The rate of growth and development of the microsporidian in the body of the larva is remarkably rapid The larvæ are healthy when young and infection usually takes place during the first three or four days of its life On the 6th or 7th day there is a marked translucency of the tissue in the region of the infection and by about 12 days a considerable portion of the body has been invaded In most cases it has been found that the development of the imaginal organs like the wings and legs is arrested and they are to be seen as nothing more than mere rudiments As such these larvæ do not pupate at all A comparison of a section of a healthy larva with that of a parasitized larva would reveal the changes brought about by *Thelohania* infection While in the healthy larva the imaginal parts like the wings halteres and legs are well developed the pupal breathing siphons well formed and the fat body healthy in the parasitized larva the imaginal parts are absent or only rudimentary the pupal siphons are absent, and the fat body replaced by masses of the microsporidian colonies (Plate VIII fig 2) The larvæ die without undergoing any further development and on the disintegration of the body the spores are liberated into the water and fresh infection occurs when they are swallowed by healthy Anopheline larvæ

The spores of *Thelohania* from different host species of Anopheles larvæ vary markedly in regard to size and as mentioned previously, it is probable that they belong to more than one species

The measurements of the spores are given below —

Host	Measurement of spore
A barbirostris	4 5 to 4 9 μ by 2 7 to 3 μ
A varuna	3 6 μ by 2 3 μ
A hyrcanus	4 5 μ by 2 7 μ
A pseudojamesi	4 5 to 5 4 μ by 3 2 to 3 6 μ
A fuliginosus	3 6 to 4 5 μ by 2 3 to 2 7 μ

The above measurements are of fixed and stained material Fresh spores measure slightly larger Some shrinkage is caused during the process of drying and staining

The life cycle of the species of *Thelohania* infesting larvæ of *Anopheles pseudojamesi* has been studied in detail (Text Fig 1) The edge of the parasitized region is the area usually rich in vegetative forms and a smear of the crushed fat body of this region is helpful in studying the vegetative forms The youngest cell is a small form with a compact densely stained nucleus and well stained cytoplasm This cell grows larger the nucleus divides the cytoplasm gets constricted and two daughter cells are formed This is the schizogonous cycle of the parasite The sporogony cycle starts from one of these binucleated schizonts which undergo a further division of nuclei without a cytoplasmic division with the result that a form with four large nuclei is formed This now divides into two bodies each with two nuclei Fusion takes place of the two nuclei within the cell and the product of this fusion is the sporont The sporont

grows to be much larger the nucleus undergoes three consecutive divisions, and the result is a pansporoblast with eight sporoblasts A further stage is a group of eight spores enclosed within a thin envelope The spores germinate by extruding the polar flagellum when taken into the stomach of a new host larva the young infective form emerges out and thus completes the life cycle

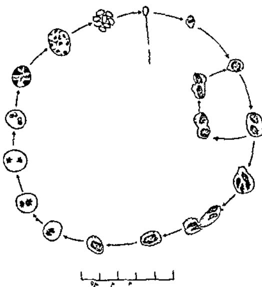


Fig 1 Life history of *Thelohania* sp parasitic on the larva of *Anopheles pseudjamesi* Original

In many ways the life history of this species of *Thelohania* is similar to that of *T legeri* which has been excellently worked out by Kudo This parasite infests the larvæ of certain Anophelines in France and in America Kudo (1924, pp 32 33) gives the following description of its life history — The youngest stages found in the infected fat body cells of the host are rounded bodies with compact chromatin granules (Text Fig 2 *1*) The nucleus becomes vesicular and then divides (*2* to *5*) At the same time the cytoplasm becomes constricted and finally two uni nucleated daughter schizonts are formed (*6* and *7*) This division is repeated Some of the schizonts which contain two daughter nuclei (*5*) remain without cytoplasmic division and grow in size In the meantime each of the two nuclei undergoes division simultaneously so that a large elongated body with four nuclei is produced (*8* and *9*) Division takes place and two large schizonts are formed each with two nuclei (*10*) These nuclei lose their vesicular nature, become compact and divide the daughter halves being very often connected with each other by a strand (*12*, *13*) By division two binucleated forms are formed ,

the nuclei are cousin nuclei and not daughter nuclei (*14*) The zygote or sporont is formed by the fusion of the two nuclei (*15* and *16*) The nucleus now divides three times in succession producing stages with two, four and eight nuclei (*17* to *2*) Now the sporont is transformed into a pan sporoblast with eight sporoblasts each of which develops into a spore When the spore reaches the gut of a new host larva the filament extrusion and emergence of the sporoplasm as an amœbula take place and the development is repeated'

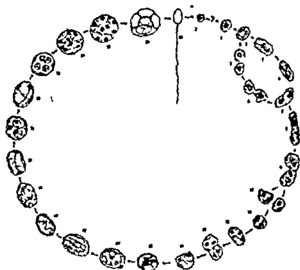


Fig 2 Life history of *Thelohania legeri* (From Kudo 1924)

It may be pointed out that there have been very few records of microsporidia from India and I may say that not even a single species of *Thelohania* has been properly recorded The only microsporidian known are three species of *Nosema* * one of which *N bombycis*, is of economic importance as it causes the pebrine disease of silk worms But there has been no reference anywhere to *Thelohania* with the exception of a vague mention by Ross (1906) of 'The spores of a protozoan parasite consisted of a collection of exactly eight spores closely packed within an oval envelope' This is probably a reference to some species of *Thelohania* This was from a species of Stegomyia and from *Culex fatigans* in the nerve chord of the adult mosquitoes Other than this very vague mention of spores grouped together in groups of eight, there has been no record of this genus and none from any species of Anopheles from India

Further work is being done with regard to the determination of the *Thelohania*

1 *Nosema bombycis* Nageli, 18
2 *Nosema ctenocephali* Kudo, 1924 Syn *N pulicis* Korke, 1916
3 *Nosema adiei* (Christophers, 1922) Shortt and Swaminath, 1924

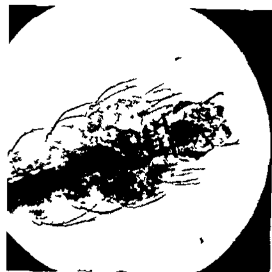


Fig 1

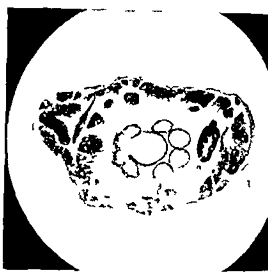


Fig 2

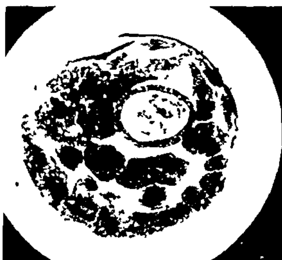


Fig 3

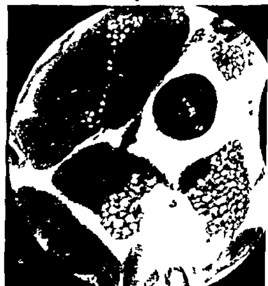
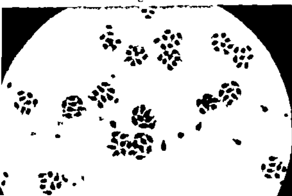


Fig 4





EXPLANATION OF PLATE VIII

Fig 1 Photo micrograph of a larva of *Anopheles varuna* parasitized by microsporidia of the genus *Thelohania* Note the opacity of the thorax and abdominal segments

" 2 Transverse section through the thorax of a diseased larva The fat bodies have been thoroughly invaded by the parasites The wing rudiments are very poorly developed

" 3 Transverse section through the abdominal region of a larva parasitized by *Thelohania* The fat cells are totally invaded by the parasites and the *body is considerably swollen up*

" 4 Photo micrograph of a section through the thorax of an Anopheles larva in the earlier stages of parasitization The section shows the fat bodies being slowly invaded by the microsporidian The densely stained portions are full of parasites whilst the yet uninvaded portions are healthy looking

" 5 Photo micrograph of *Thelohania* sp from the larva of *Anopheles varuna* showing mature sporoblasts with eight spores each and also individual spores scattered in the field

" 6 Photo micrograph under dark ground illumination of the polar flagellum of *Thelohania* capsules which were exploded by mechanical pressure The basal portion of the flagellum is much thicker than the apical portion

of the first longitudinal vein, basal half of the latter mottled with creamy white scales, anterior branch of the second vein with the apex creamy white, a similar spot toward the base of the posterior branch, apex with pale spot, third longitudinal vein with a creamy patch toward the apex, rest of the vein mottled, anterior branch of the fourth vein with a patch of creamy scales, mottled toward the apex, posterior branch mottled, anterior branch of the fifth vein with an apical and basal creamy spot, rest of vein mottled, sixth vein usually with one prominent creamy apical area, fringe with pale areas where the veins join the costa except at the junction of the posterior branch of the second, third and sixth veins, halteres with pale stems and black knobs

Legs with the femora, tibiæ and first tarsi densely mottled with pale creamy white and black scales, the former predominating except on the mid and hind tarsi the second tarsal segment of the fore legs with a pale apical spot, first and second tarsi of mid legs with pale creamy white apical rings tarsi one to five of hind legs with basal and apical pale creamy white banding, ungues equal and simple

♂ Palpi black scaled, apex of basal third with a yellowish ring apical third spatulate and covered with moderately dense dusky hairs, plumes of antennæ black thorax abdomen wings and legs similar to ♀, ungues of fore legs unequal, the larger with a tooth, mid and hind equal and simple

Edwards (1924) compares the ♂ hypopygium with that of *A barbirostris* as follows —' *A bancrofti* Giles Leaflets very small and short, the longest less than a third as long as the ædœagus, the rest much shorter Basal spines of side piece on a large prominence, each with a well marked tubercle at its base, spines about equal in length and both stout, with the tip suddenly narrowed and curved Club very slender, almost like a blunt ended spine, the two spines of which it is composed sometimes partly separated *A barbirostris,* v d W Leaflets much longer and darker, two or three pairs being fully half as long as the ædiœagus Basal spines on a small prominence without tubercles both rather slender especially the outer one, which is longer than the inner and nearly straight Club stout, distinctly enlarged apically '

Length —5 6 mm without proboscis

The description of the larva and pupa is taken from Cooling (1924 *b*) and is as follows —' Larva —Length 7 mm Head and anal segment dark and heavily pigmented Antennæ slightly curved covered with many sharply pointed but simple spines the size of which latter decreases from base to apex Proximal portion of antennæ slightly swollen Antennal plume sub median in position consisting of about twelve branches, the branches are more or less equal in length and give the appearance of a radial grouping Each antenna is tipped with two stout spine like processes, weakly chitinized also with an extremely delicate branched seta of about five branches Sub antennal plume, feather like about half the length of the antenna Mouth brushes consisting of moderately chitinized simple "hairs" Labrum clothed with minute slightly recurved and distally serrated setæ Mandibles consisting of small but heavily chitinized teeth Labial

plate roughly triangular the base obtusely and symmetrically crenated there are about three lateral more or less irregular, blunt teeth and a median (apical) one of average size An asymmetrically shaped foramen occurs on either side of the median line of the labial plate The frontal hairs ' of the head exist as six well defined branched setæ Inner anterior clypeal hairs closely approximated long, stout, and very minutely branched—the branches being few and hardly more than the diameter of the hair Each of the outer anterior clypeal hairs consists of a tuft of much branching tree like hairs Thorax In addition to the usual chæto taxic grouping there is a group of three long simple hairs which arises from a moderately chitinized tubercle on the antero lateral margins of the thorax in the prothoracic region at the base of each of these hair tufts are two unequal spines one markedly chitinized the other (longer one) weakly so On the inner side of these hair-tufts are two minute branched setæ one on each side of the median line, these comprise the anterior sub median group of Stanton There is a small but pronounced feathered hair on each side of the mesothorax and a smaller and less pronounced one on the antero lateral aspect of the thorax Several very minute palmate hairs are to be found on the thorax The abdominal segments 1 to 3 have branched lateral setæ rather much like those of the thorax segments 4 to 6 with long bifid setæ All the abdominal setæ arise from distinct chitinous tubercles and in addition to the ordinary lateral setæ there are much smaller branched ones arising near their bases The typical palmate hair takes the form of rather large and mottled sharply tapering leaves the edges of which can be observed to be minutely serrated from fifteen to nineteen such leaves go to make up one of the large palmate hairs There are large palmate hairs on segments 3 to 7 minute ones (less notched) on segment 2 and still more minute ones on the thorax (two pairs) The thorax and abdominal palmate hairs show a gradual transition both in size and complexity which is a good example in the development of the typical palm ' from a simple hair tuft The spines of the pecten take the form of simple undifferentiated (except for size) spikes large roughly alternating with smaller ones The number is about fourteen in each side, and altogether the comb is very similar to that of *Anopheles annulipes* Walker The anal segment is of a mahogany colour and its surface is invested with numerous minute sharp simple spines of almost the same shape and size as those existing on the shafts of the antennæ Ventral beard arranged in about ten paired groups of typically branched hairs Dorsal beard well represented the ' grappling hooks consist of a plume of five or six branches on each side swimming fans (anal papillæ) equal moderately elongated and obtusely pointed

Pupa — A most striking feature of the pupa is the linear extensions of mottled or variegated colour markings of the cephalothorax due to the chitinous incrassations of the pupal cuticula Caudal fins rather striking by reason of their relative diminutiveness The fans are also closely approximated and do not show any tendency to lateral spreading Breathing trumpets small broad and triangular There is a pair of large tree like plumes of much branched setæ at the base of the

cephalothorax (i e on abdominal segment 1) On each of the sides of abdominal segments 2 to 6 is a short stout, bluntly *pointed and strongly chitinized spur* Each segment has a pair of branched setæ each of which branches from a short stalk into five more or less equal and regularly disposed hairs" There are also minute bifid and trifid ' hairs" on each segment The seventh segment bears a pair of small plumes and peculiarly branched hairs on the posterior angles The leaves of the caudal fin are hyaline each stiffened in the ordinary way by a midrib which latter is very faintly striated transversely and terminates in a short chitinized spur or bristle '

Habitat —Queensland North Australia, territory of New Guinea (Madang and Admiralty Islands), Philippine Islands, Ceylon (Strickland)

Breeding places —*The larva of this species avoids localities subject to direct* sunlight moreover it has never been found in small collections of water such as hoof holes of animals generally being found in moderately shallow water overgrown with vegetation Larvæ of this species have also been found in slowly running water

Habits —The adults taken by the writer have in every instance been captured in situations where direct sunlight did not penetrate They rarely frequent houses To the writer's knowledge it will bite freely from 2 until after 11 P M It is a gross feeder and a most vicious biter, ' striking ' as soon as it alights and will continue to suck blood though apparently fully gorged passing tiny droplets of a clear fluid *per annum until its abdomen is so replete with blood that it is* unable to fly more than a few feet without resting

A bancrofti is very closely related to *A barbirostris* and has been placed by the writer (1913) as a variety of the latter Edwards (1924) states that '*A barbirostris* however shows differences in the hypopygium which are almost certainly of specific value ' On the other hand the adults of *A bancrofti* are closely related also their habits and breeding places are very closely related to those of *A barbirostris*

Christophers (1924) and Covell (1927) give *A pallidus* Swellengrebel and Swellengrebel from the Dutch East Indies as a synonym of *A bancrofti* Giles but a specimen of the former in the Institute Collection determined by Dr Brug differs especially in the leg markings from *A bancrofti*

Christophers (1922) records *A umbrosus* as from Australia but the writer considers this record is in reality meant for *A bancrofti* especially since Christophers (1924) has not repeated the statement

Relation to Malaria —There is no evidence that this species is a vector

Anopheles (Anopheles) stigmaticus Skuse

Proc Linn Soc N S Wales (2) Vol III p 1758 1888 *corethroides* Theob Mon Culicid, Vol IV, p 35 1907, Edwards *Bull Ent Res* Vol XIV p 35. 1924

♀ Head yellowish brown clothed with narrow yellowish upright scales and hairs the latter numerous in the centre and overhanging the eyes antennæ brown basal lobe dusky brown second segment pale at the base palpi brown clothed with dusky scales slightly shorter than proboscis latter brown

Thorax greyish brown with a broad dark brown median area extending from the anterior margin to about the middle of the scutum where it broadens out to the lateral margins and extends to the posterior margin covered with yellowish brown hairs scutellum brown border bristles yellow prothoracic lobes prominent pleuræ dusky brown

Legs brown covered with violet brown scales hind femora with the basal four fifths pale yellow

Wings costa black veins clothed with brown scales first fork cell longer and narrower than the second base of the former nearer the base of the wing than that of the latter stem of the first fork cell about the length of its cell stem of the second longer than the cell anterior and anterior basal cross veins parallel the latter slightly less than its own length from the former a dark patch covering the forking of R and Rs

Abdomen dark brown sparsely covered with golden hairs denser at apex venter brown

♂ Similar to ♀ Antennæ brown plumes brown and very dense palpi brown slightly shorter than proboscis last segment spatulate and clothed with short brown hairs concerning the hypopygium of the ♂ type of *corethroides* Edwards (1921) states that it is in some respects rather peculiar It has only one basal spine on the side piece which is set on a distinct tubercle (as in the Palæarctic *A algeriensis* Theo) The claspettes are conical pointed not lobed with one long slender bristle at the tip and one shorter one below it the ædœagus is moderately long and slender with about six pairs of very long and slender leaflets To this Mackerras (1927) adds that the genitalia of both northern and southern forms agree with Edwards description of the type of *A corethroides* Theo There is no doubt that the synonymy given by Edwards is correct but the typical *corethroides* form is distinctly less well marked than typical *stigmaticus*

Length —4 5 to 5 mm

Larvæ dark brown and superficially resemble small specimens of *A annulipes* Walk marked eye separation of the two being impossible The antennæ are creamy darker at the tip and bear apically two spines and a single fine hair which is forked distally the antennal hair is situated at the junction of the basal and middle thirds of the antennal length and is very short and finely branched The head is uniformly dark brown in colour and both compound and simple eyes are present The inner anterior clypeal hairs are long bare and set close together the outer anterior clypeal hairs are a little more than half the length of the inner and are bare the posterior clypeal hairs are short and single or divided into two or three very terminal branches The frontal and sub antennal hairs are normal The inner occipital hair is bifid and the outer lies well anterior to it and is trifid The

chætotaxy of the thorax and abdomen is not unusual Palmate hairs occur on segments 2 to 7 of the abdomen are well developed and conspicuous and each consists of 20 to 25 leaflets which are long slender, pointed, and not serrated The pecten is composed of regularly alternating long and short teeth The ventral sub dorsal hairs bear the usual grappling hooks

The pupal skin is a clear pale brown and the trumpet is not pigmented nor are there any zones of pigmentation on the cephalothorax This stage may usually be separated from that of *A annulipes* Walker, by its smaller size and paler colour The trumpet is longer and not so broad, and the abdominal chætotaxy is not so striking From *A atratipes* Skuse it may be distinguished by its smaller size, absence of pigmentation, much narrower trumpet and different chætotaxy The pupal stage lasted two to four days under the experiments

Habitat —S Queensland Burpengary Brisbane, N S Wales, Blue Mountains, National Park (Near Sydney)

This a very distinct species, the hind femora being very conspicuous It cannot be confused with the other Australian Anophelines Mackerras (1927) states that the resting attitude is almost horizontal and the palpi are carried closely appressed to the proboscis, so that they resemble when alive a small species of *Culex* much more than an Anopheline Northern and southern specimens vary somewhat *inter se* but they entirely conform in specific characters

The larval and pupal descriptions have been copied *in toto* from Mackerras

Relation to Malaria —No evidence It is extremely improbable that this species would ever play any part as a vector on account of its extreme rarity

Anopheles (Anopheles) atratipes Skuse

Proc Linn Soc N S Wales (2), Vol III, p 1755, 1888

♀ Head covered with white upright forked scales and similar black ones laterally, a conspicuous tuft of long white and creamy hairs overhanging the eyes from the centre, antennæ brown, verticillate hairs brown, basal lobe dusky, second segment about the length of the third, palpi brown with outstanding dusky scales on the basal third, appressed brownish ones on the apical two thirds, proboscis black

Thorax black with a broad median reddish brown stripe from the anterior margin to the middle of the scutum sparsely clothed with small pale scales and dark hairs, scutellum pale creamy yellow, the centre black, sparsely covered white narrow scales, border bristles dusky, pleura *yellowish brown, darker posteriorly*

Abdomen black covered with dense yellowish brown hairs

Wings costa subcostal and first longitudinal vein densely clothed with dusky brown scales There are patches of dusky brown scales at the bases of the fork cells, at the base of the third, and at the base of the fork of the fifth, the sixth vein is covered with pale scales except for about the apical fifth, there is a patch of white scales in the middle and a small white spot near the base of the third the

upper branch of the fourth has the apical two thirds white the middle third of the posterior branch is white the vein is also lightly mottled with white scales for a short distance posterior to the base of the fork the fifth to the base of the fork cell is white scaled with a prominent patch of dusky scales in the centre both branches of its fork cell are mottled with brown and white scales

Legs covered with dark brown scales

♂ Palpi with the terminal segment swollen and with a patch of long hairs ventro laterally on the basal half of the terminal segment and extending on to the apical part of the penultimate segment antennæ with long dense silky brown plumes, otherwise resembling the ♀ in all essentials The hypopygial characters are in some respects intermediate between *A bancrofti* Giles and *A stigmaticus* Skuse but show much closer affinities with the latter in the single basal spine of the side piece in the pointed rather than lobed claspettes and in the replacement of the club by a row of four basal spines set on the lobe It resembles *A bancrofti* Giles in bearing three sub equal spines on the claspette There are about two pairs each of longer medial and shorter lateral leaflets

The larvæ are darker than those of *A annulipes* Walker but do not afford any naked eye characters of value for differentiating them The antennæ are brown notably stout and short and bear apically two spines and a fine trifid hair The antennal hair is situated slightly basal to the middle is fully half the length of the antenna and is strongly branched The head is very heavily and uniformly pigmented and no trace of eyes could be made out in any of the preparations The anterior clypeal hairs resemble those of *A stigmaticus* Skuse but the posterior are very short and single The frontal hairs are strongly plumed and are sub equal in length The sub antennal plume is normal The inner occipital hair is divided into four very fine branches The outer lies well anterior to the middle and is trifid The thoracic and abdominal chætotaxy is not remarkable except for the entire absence or great reduction of the palmate hairs These structures may be represented by certain short hairs on a few of the abdominal segments but are certainly not present in the form seen in other Anopheline larvæ The pecten consists in general of long spines alternating with two very short ones Grappling hooks are developed

The pupa appears to be closest to Cooling s description of *A bancrofti* Giles The skin is brown with strong lines of black pigment on the wing sheath along the course of the veins and with transverse dark bars in a row along the antennal sheath The trumpet is very broad triangular in shape and is deeply pigmented

Habitat —Queensland Stradbroke Is Caboolture to Enoggera N S Wales Berowra Sydney district

This species bears a superficial resemblance to *A bancrofti* Giles but the clothing of the palpi wing and leg markings render it easily separable It is similar in habits in that it is a sylvan day biting species (Mackerras) and rests at an angle of about 80 per cent with the surface It is most prevalent in the coastal districts of N S Wales and South Queensland in the spring and early summer, apparently

disappearing completely in the season when other Anophelines are most abundant In the Sydney district it occurs most frequently in the sandstone gullies and in the vicinity of sluggish creeks running through the Pleistocene sand flats

It is a vicious biter causing considerable pain

The larvæ were taken in a *small sluggish slightly muddy creek with a fine* silt bottom The pH of a sample of the water was 7 5 Some aquatic vegetation was present but not a great deal Other mosquito larvæ were entirely absent

The affinities of the larvæ are with A stigmaticus Skuse from which it is to be separated by its larger size much darker colour much stronger antennal plume and by the absence of eyes and palmate hairs

Relation to Malaria – There is no evidence and very probably would never play any part in the role of a vector on account of its rarity

Anopheles (*Myzomyia*) *annulipes* Walker

Ins Saund Dipt i p 433 1856 *musivus* Skuse Proc Linn Soc N S W (2) Vol III p 1751 1888 *mastersi* Skuse op cit p 1757 1888

♀ Head dusky clothed with pale scales and dusky ones laterally a tuft of *white hairs projecting over the eyes from the centre* antennæ brown *first two* segments with small white scales pubescence pale verticillate hairs dark proboscis with slightly more than the basal half blackish brown remainder creamy white palpi with segments two to four with broad white apical bands

Thorax grey black clothed with numerous pale creamy white scales and scattered pale hairs scutellum dusky, white scaled marginal hairs long dark

Abdomen dusky brown densely clothed with yellowish hairs segments two to seven with narrow pale creamy white scales most numerous on segments six and seven

Wings with the veins covered with conspicuous patches of black and cream coloured scales the costa with three small cream and two small black basal patches followed by a larger black and a smaller cream one then a black one about twice the length of the previous black one in the centre of the costa followed by cream and black ones of about the same length then a small cream one and a longer black one the first long black patch of the costa extends to the subcostal and first long vein the second embraces *the subcostal the third and fourth embrace the* first long vein, the junctions of the veins with the costa are creamy first fork cell *longer and narrower than the second* base of the former nearer the base of the wing than that of the latter

Legs covered with dark brown scales femora and tibiæ with numerous white rings tibiæ of mid and hind *legs with apical white banding first tarsal* of fore legs with basal and apical banding and a broad white band in the middle which has a small brown patch in its centre second tarsal with basal and apical banding remaining tarsi of fore legs unbanded, tarsi *of mid legs unbanded first tarsal* of hind legs with two narrow white bands about the apex of the basal fifth then follow

six small white spots about equally divided in distance along the remainder of the tarsus tarsi one to four with basal and apical banding the fifth with basal banding only

♂ Palpi apex of basal third with a narrow white band likewise the base of the middle third apical third spatulate with numerous pale hairs base black scaled remainder white scaled above with a small median black spot antennæ with dense light brown plumes

Side piece of hypopygium bears four basal spines The leaflets four or five in number and rather broad about half the length of the ædœagus claspette rounded with a long apical hair and a single accessory one which is at least half as long as the apical one This is a variable character and Mackerras (1927) says the length of at least one or two of the accessory hairs is constantly as much as half the length of the main hair in all specimens examined an important point of distinction from *A amictus* Edwards in which these accessory hairs are never more than a quarter the length of the main hair

Length —4 5 mm

Head of larva brown with the pigment arranged in a definite and characteristic pattern antenna with a short sub lateral spine about its middle length inner anterior clypeal hairs well apart much nearer the outer anterior clypeal hairs than to each other sometimes bearing two or three extremely short fine plumes outer anterior clypeal hairs about two thirds the length of the inner and strongly plumose posterior clypeal hairs are wider apart than the inner anterior are four branched short and not projecting as far as the anterior border of the head inner occipital hair trifid the outer with a few plumes leaflets of palmate hairs resembling the shape of a cabbage palm leaf (Cooling 1924a) pecten variable with three long teeth

Pupal skin brown without specially pigmented areas except the trumpet which is lightly powdered with pigment the stout lateral spines of the abdomen decrease in size proximally and are very short on the basal segments The trumpet is oblong in shape and slightly shorter and broader than that of *A stigmaticus* Skuse (Mackerras 1927)

Habitat —Queensland Cairns to Brisbane N S Wales Victoria Tasmania South Australia Southern West Australia Central Australia Alice Springs Charlotte Waters

This species has been recorded from several localities to the north and west of Cairns It is most probable that these records belong to *A amictus* Edwards those in North Australia and the north west of West Australia also most probably refer to *A amictus* Edwards

It is a variable species and closely related to *A amictus* Edwards

Nicoll (1918) writes of *Anopheles maculipennis* as being present in North Queensland This is quite erroneous and no credence whatsoever can be placed on his entomological statements His remarks refer to either *A annulipes* or *A amictus*

Habits —This species bites at all times of the day and night In Victoria where the writer (1917) found this species very abundant they were found to bite most freely at sunset and for about an hour afterwards Adults enter houses quite freely

Breeding places —The breeding places *par excellence* are hoof marks of cattle and horses containing either muddy or clear water without vegetation, other breeding places are grass grown channels small casual collections of water street gutters containing slowly running water Large expanses of even shallow water do not seem to be favoured as a breeding ground

Relation to Malaria — There is no conclusive evidence, it is entirely presumptive though possible on epidemiological grounds (Breinl and Taylor 1918 Money 1926)

That this species does not occur in Formosa has been definitely proved by Koidzumi (1924) and Yamada (1925) therefore the work of Kinoshita (1906) fails in relation to *A annulipes* the species with which he worked being established as *A tessellatus* Theob

Anopheles (*Myzomyia*) *amictus* Edwards

Bull Ent Res, Vol XII, p 71, 1921

A larger and more yellowish species separated in the ♀ from *A annulipes* by an entirely dark proboscis and numerous moderately broad scales on segments two to seven of the abdomen and in the male by its hypopygium the main characteristic being the presence of two or three fine short hairs which are always less than one quarter the length of the main hair The scales on the abdomen in both sexes are broad flat and creamy yellow in colour Hill (1925) states that he bred typical ♀ *A annulipes* Walker among the progeny of *A amictus* Edwards females This the writer is inclined to believe was due to the presence of a true ♀ *annulipes* unknown to Hill If not then there is a most complex question to settle for the larva of the true *A amictus* Edwards is very different from that of the true *A annulipes* Walker

For this reason the writer submits no detailed description of A amictus Edwards until he or another worker has definitely established the validity of *A amictus Edwards*

The larva differs from that of *A annulipes* Walker, in the following respects (Mackerras 1927) The *sub lateral spine of the antenna is definitely beyond the* middle the outer anterior clypeal hairs are single (unplumed) and the posterior clypeal hairs are single or sometimes bifurcate and extend well beyond the anterior margin of the head, the inner occipital hair is single and the outer is trifid the *comb differs slightly in the arrangement of the* long spines but is variable, in other respects including the palmate hairs there is little or no distinction '

Habitat —Queensland North Australia north western area of W Australia

Habits —The biting and resting attitudes of this species are similar to those of *A annulipes* Walker so far as the experience of Mackerras and the writer goes

Breeding places —These all appear to agree with those of *A annulipes* Walker, the two being found alongside of one another

Though there is as yet no data the writer believes that this species will eventually be found in northern and western N S Wales

Relation to Malaria – There is no evidence but it is possible that it may play a part as a vector since the writer (Breinl and Taylor 1918) certainly confuse it with *A annulipes* Walker in the survey of Cairns

Anopheles (*Myzomyia*) *punctulatus* Donitz

Insectenborse Vol XVIII p 372 1901

The main distinctions of the type form from its variety *moluccensis* may be given as follows —The white scales on the head are mainly confined to the fore part all the rest being black palpi apex of basal third with a narrow white ring rest black and somewhat shaggy apex of middle third with a narrow black ring and just basal to it a narrow white ring rest black base with black ring elsewhere white except for a sub apical black ring proboscis with the basal two thirds black, apical third white scaled except for a narrow black apical ring labellæ reddish brown ground colour of thorax and scutellum greyish black femora tibiæ and first tarsi not so profusely marked with incom plete rings and the tarsi are unbanded the costa of the wing has three small yellow spots basally then a much larger black one which extends to the first long vein followed by a small yellow spot which expands on to the subcostal and first long veins this is followed by a long black patch which includes the subcostal vein then a small yellow patch followed by a black patch slightly shorter than the preceding black one followed by a fairly long yellow patch, occupying a position about the middle of the first fork cell a similar black one about the length of the last yellow one follows which embraces the first long vein the veins are much more evenly mottled there being fewer yellow scales than in var *moluccensis* on the veins especially is this noticeable on the sixth which has the base black scaled then follow three small black and three small yellow patches alternately to about the apical fourth which is black scaled except the extreme apex which is yellow the basal half of the anterior branch of the fifth is black scaled there is a similar patch about the same length extending from the base of the fork towards the base of the vein

The above data are taken from a ♀ specimen kindly presented to the Institute by Dr S I Brug the label bearing the following *A punctulatus* Don nec Theo Pionierbivak (N Guinea) early in 1922 as the Institute does not possess speci mens of this species from New Guinea or Australia

Habitat —N Australia Borroloola Papua Moresby Lakekamu Goldfield, Mekeo district Siriba Cape Nelson Samarai Is Woodlark Is territory of

New Guinea Rabaul, Toma, Beining district, Dutch New Guinea Pionier bivak, Kaimana, Moluccas, Solomon Is, New Hebrides ? Santa Cruz Group Vanikoro Is (a specimen from Dr Deland was damaged in the post and could not be identified with certainty)

Breeding places —Heydon (1923) records the larvæ from New Britain in temporary puddles and shallow pools of rain water, often muddy but not foul in hoof marks or in local depressions of the ground in localities where the soil is not too porous to retain such water Buxton (1926) and Covell (1927) found them in swamps and stagnant surface water in the New Hebrides

Habits —The adults are found in houses and feed throughout the night Breeding occurs throughout the year

Relation to Malaria —Heydon (1923) infected this species experimentally with M T parasites (sporozoites in two specimens out of seven dissected) He notes that it was uncertain whether the five remaining specimens had fed

Covell (1927) states that De Rook found oocysts in 12 specimens out of 418 dissected in nature in New Guinea (2 9 per cent) and considers the species to be an important vector Covell also states that Buxton considers that *A punctulatus* is responsible for the malaria in the New Hebrides, as it is the only species recorded in the whole of Melanesia

The record of this species from Borroloola, N Australia, by Hill (1925) is very interesting and exceedingly important from the aspect of malaria epidemics and has been discussed under *A punctulatus* var *moluccensis*

Anopheles (*Myzomyia*) *punctulatus* Donitz, var *moluccensis*

Swell and Swell, Bull Ent Res, Vol XI, p 78, 1920

♀ Head clothed with white upright scales, with some whitish hairs overhanging the eyes from the centre, lateral scales brown Antennæ light brown, basal segment with small white scales, pubescence and verticillate hairs white, proboscis dark brown, a few dark brown outstanding scales at the base, more pronounced ventrally, apex pale yellow, palpi first segment dusky brown with white apex, second segment dusky brown with a broad white band on the apical half, its apex with a narrow yellowish one, penultimate and apical segments with a narrow dusky brown basal band, the remainder creamy yellow

Thorax greyish (ground colour apparently brown) with a dark spot on each side about one third from the anterior border, one in front of the scutellum and another small one in the centre on the apex of the anterior third, clothed with fairly dense white narrow scales and sparse yellowish hairs, scutellum pale, centre brownish clothed with white narrow scales, border bristles yellow, pleuræ brown to dusky with a few pale narrow scales, halteres white, apex of knob black scaled.

Abdomen dark brown clothed with numerous pale yellow hairs last three segments with numerous narrow yellow scales apex with a few black ones in addition

Legs dark brown femora tibiæ and first tarsi with numerous incomplete white rings tarsi one to three with apical and basal banding fourth with basal banding fifth unbanded in the hind legs the fifth is apically pale fourth and fifth tarsi of mid and hind legs pale ventrally

Wings with four long black patches on the costa and three short ones basally a short black spot between the first and second long ones the second to fourth long patches (from the wing base) extend to the subcosta all the longitudinal veins mottled with alternating patches of pale yellow and black scales there are three moderately long pale yellow patches on the apical half of the costa apical half of the third vein pale yellow a long pale yellow patch about the middle of the anterior branch of the fifth posterior branch with three small regularly spaced black patches the first sub basal the second almost central the apical one about its length from the apex sixth vein pale scaled with four small patches of black scales the first and fourth not quite basal and apical respectively the remaining two equally spaced the costa at the points of junction of the veins pale yellow elsewhere the fringe is dusky base of first fork cell near the base of the wing than that of the second fork cell stem of the latter about the length of its cell stem of the former more than twice the length of the cell anterior basal cross vein about twice its length from the anterior

♂ Palpi with first segment dark brown mixed with white scales slightly less than the apical half of second white scaled above with a narrow dark brown ring in its middle some pale apical forwardly projecting hairs ventrally club with the basal half white scaled except for a narrow brown basal ring another similar ring in the middle rest of club yellowish

Length —5 mm

Habitat —North Australia Darwin to Katherine territory of New Guinea Rabaul Madang Admiralty Islands Papua Mekeo district Lakekamu Gold field (Central Division) Dutch New Guinea Moluccas

Habits —This variety frequents houses where they are to be found during the daytime The females bite throughout the night They rest on the walls often near the ground on the undersides of beds on wooden floors near the walls and in sheltered and usually dark situations generally In places visited by the writer (Heydon 1923) this species never bites by day or even at early dawn or early evening but it is said to do as in localities where it is very numerous

Breeding occurs throughout the year the adults becoming more numerous after the onset of the wet season and scarcer as the dry advances

Larvæ are found in swampy pools and ditches They appear to be indifferent as to whether the water is fresh or brackish polluted or otherwise natural or artificial

Relation to Malaria —Heydon (1923) at Rabaul infected this variety with B T parasites (7 out of 15 dissected) and with M T (7 out of 7) In nature he found a total sporozoite rate of 3 9 per cent (206 specimens) and a total oocyst rate of 3 6 per cent (220 specimens), the sporozoite and oocyst rates among specimens taken in native quarters being 6 4 per cent and 6 2 per cent respectively (a small proportion of specimens dissected probably belonged to the type form) De Rook (Covell, 1927) also considers that this form is an important carrier of malaria

The writer after mature consideration considers that this form has been in the past, responsible for the epidemics which have occurred in North Australia since Hill (1925) definitely states that it is common between Darwin and Katherine which being so it probably extends throughout N Australia It is also suggested here, that this mosquito has, perhaps with the type form, been responsible for the severe epidemics which occurred on the Palmer Goldfield in the early 'eighties It is admitted that neither this form nor *A punctulatus* have ever been recorded from Cape York Peninsula, but against that it has to be borne in mind that what few Anophelines have been taken on the far distant parts of this peninsula have not been critically examined by a competent authority and no longer exist The writer considers that when the shaded area on the Map (*see* end of paper) is carefully searched, under favourable conditions, either or both of the above-mentioned mosquitoes will probably be found there

Anopheles (*Myzomyia*) *subpictus* Grassi

Atti d R Acad d Lincei, Ser 5, Vol VIII, sem 1, fas 3, p 101, 1899 (Feb), *rossi* Giles Jnl Trop Med and Hyg, Vol II, p 63 1899 (Oct), Edwards, Bull Ent Res, Vol X, p 129, 1920

♀ Head blackish with pale scales in front, and with a tuft of pale hairs projecting forwards, black scales at the top and sides, eyes black, antennæ brown with pale hairs and pubescence, basal joint ochraceous brown with a few creamy scales, proboscis dark brown, apex sometimes pale, palpi dark scaled, apically white, and with two other pale bands near the apices of the second and third joints, clypeus pale brown

Thorax pale yellowish brown to ochraceous brown with greyish reflections with traces of a median line, covered with scattered pale scales and hairs, scutellum pale sometimes dusky in the middle, with pale scales and brown bristles much as in *superpictus*, metanotum pale yellowish brown to brown, pleuræ with a dark line of spots above pale below, with patches of pale scales

Abdomen dusky, densely clothed with golden brown or ochraceous hairs which are especially thick at the apex giving it an ochraceous appearance

Legs yellowish with brown scales and with pale apical and basal bands to some of the tarsi except the last tarsus, which is always black The tarsal scales are very dark sometimes having a deep purplish brown hue Fore tarsi basally and apically banded yellow, except the last joint, metatarsus apically banded

only in some specimens there may be seen a very narrow basal as well as apical banding to the hind legs

Wings yellowish with the costa broken by four large patches of dark brown or black scales and two or three smaller basal ones The large middle spot has a small dark spot below in the centre giving a T shaped appearance to it First sub marginal cell a little longer and narrower than the second posterior cell their bases nearly level with one another that of the first sub marginal if anything nearer the base of the wing The stem of the second posterior cell about the same length or a little longer than the cell The posterior cross vein nearly twice its own length distant from the mid cross vein Scales on the veins creamy yellow with small black patches as follows at the tips of all the veins one on each branch of the first fork cell one at the base of the third long vein two on the upper and one on the lower branch and two on the stem of the fourth vein three on the upper branch one on the lower and one near the base of the stem of the fifth vein two spots on the sixth long vein of small size Fringe yellow at the apex and at the ends of the fourth and fifth veins remainder blackish

♂ Palpi swollen at the end yellow with a broad black band at the base a broad black band in the middle and a small broken one near the apex of the same joint and a narrow ring of black at the base of the last two joints hair tufts short pale the base of the palpi densely black scaled proboscis dark brown pale at the tip antennæ with silky golden brown plumes Wings marked much as the ♀ but in many ♂'s especially in those from South India there is a small additional spot beneath the second costal spot besides the one forming the T and in a few ♀'s I have noticed the same First posterior cell a little longer and much narrower than the second posterior cell its base if anything a little nearer the apex of the wing than that of the latter its stem equal to the length of the cell the same length as that of the second posterior cell supernumerary cross vein nearly its own length in advance of the mid cross vein posterior cross vein at least twice its own length distant from the mid cross vein fore ungues unequal the larger one twice toothed mid and hind equal and simple the mid rather the longer

Length —4—6 mm

Habitat —Papua near Port Moresby Mekeo district (60 miles west of Port Moresby), India Malaya Siam Dutch East Indies Philippine Islands

Theobald s description of *A rossi* has been utilized as the only Papuan specimen a ♀ taken by Hill in the Port Moresby district in the Institute Collection lacks the abdomen and one wing The head thorax and remaining wing are, however quite typical the legs are all abraded

Breeding places —The larvæ are found in pools often muddy rain pools and in almost any temporary or permanent collection of water frequently heavily contaminated with sewage The breeding places are most frequently found in the vicinity of villages (Covell 1927) Hill (1925) found the larvæ in large numbers in beached native canoes within easy flight of a large native village and the official

residential area of Port Moresby The identification was made by Edwards from specimens sent to him by Hill

Habits —The adults of this species are found in large numbers in cattle sheds and in human habitations and feed readily on man even if cattle are present (Covell 1927)

Relation to Malaria — This species has been experimentally infected with all three forms of the malaria parasite Hill (1925) states there is the strongest circumstantial evidence that it is the species responsible for an infection of benign tertian malaria contracted by the writer, and it is possibly *an important factor* in the transmission of the disease in that portion of the possession where canoes have not been suspected hitherto of affording a breeding place for *Anopheles* Against this statement it must be remembered that Hill was in malarious districts both in Papua and the territory of New Guinea where he may have contracted the infection such lying dormant until after he had left the Port Moresby district since the part played by this species in the transmission of malaria is extremely doubtful as the malaria parasite has never, in India at least been found in wild dissected specimens Covell (1927) says this species has however been found infected in the Dutch East Indies, though it may perhaps be doubted whether the *A rossi* referred to is identical with the Indian form He further states that Rodenwaldt and Essed conclude that malaria there (Dutch Indies) is transmitted by *A ludlowi* and that next to this '*M rossi* Giles,' plays a certain part as a carrier only however in the case of the epidemic being kept up by *A ludlowi* Covell gives further extensive information on the subject on work done in India

Bironella gracilis Theobald

Ann Mus Nat Hung Vol III, p 69 1905, Mon Culicid Vol IV p 121, 1907

♂ Head brown with numerous yellowish and black upright fork scales the latter very thin with bifid apex, the former broader with expanded apex with numerous serrations and apparently a few irregular narrow outstanding pale scales of similar size throughout their length

Proboscis moderately long and thin clothed with deep brown almost black scales *labellæ very acuminate palpi not quite as long as the proboscis scaled with* deep brown scales swelling gradually towards the apex the apical segment large one joint only can be detected but probably a basal one exists Antennæ brown with pale bands below the whorls of verticillate hairs hairs deep brown

Thorax dark brown with slatey sheen and with short dull golden curved hairs projecting backwards scutellum pale yellowish brown metanotum deep brown

Abdomen black nude but with black hairs narrow basally but expanded apically genitalia densely hairy Hypopygium side pieces short and stout less than twice as long as their width at the base, basal membrane large and apparently striated from the outer basal corner of each side piece arises a stout curved

finger like process which is more than half as long as the side piece, finely pubescent towards its base, otherwise bare its apical half strongly chitinized tip blunt and slightly lobed Apart from these processes there are no spines on the side pieces Claspers rather long, nearly cylindrical with a post median hump on either side terminal claw short and broad Ninth tergite a narrow transverse oval, posterior margin simple Anal segment membranous elongate conical two thirds as long as the side piece Aedœagus (theca) a long slender, cylindrical tube with a single pair of reflexed leaflets at its tip, the leaflets a little over a quarter as long as the tube

Legs long and thin, brown covered with small scales the coxæ and under side of femora pale ochreous Ungues apparently all equal and simple

Wings with brown scales The subcostal cell tinged with brown rest of the membrane transparent, the first sub marginal cell very small its stem more than four times the length of the cell, the posterior cell about two and a half times the length of the former and about twice its width stem of the second posterior cell a little longer than the cell, curved about its centre the third long vein also curved continued to the base of the wing as a distinct pseudo vein fifth vein with its upper branch distinctly waved after its junction with the posterior cross vein sixth long vein nearly straight until its apex, where it curves abruptly a distinct pseudo vein between the fifth and sixth marginal cross vein very long and prominent, the supernumerary very small the mid as long as the marginal joining the supernumerary, the posterior not as long as the mid and close to it Halteres with the pale stem much swollen basally constricted apically the knob blacks

♀ Proboscis black straight labella dark grey *Palpi black with grey hairs at the apex, the apical fourth slightly swollen length nearly equal to two thirds* of the proboscis The segments of the palpi are scarcely distinct even with the specimens mounted in balsam and magnified × 300

The length of the palpi in the ♀ is found to be 60 per cent 64 per cent 64 per cent, 65 per cent 66 per cent 66 per cent and 67 per cent of that of the proboscis

Antennæ composed of 14 segments of a dark greyish brown without scales with short grey hairs equally distributed whorls of hairs black at each joint with the exception of the basal segment which is glabrous and of the second on which the hairs are irregularly placed The length of the apical segment is one and a half that of the penultimate Segments 2 to 13 are about equal in length

Occiput —*There is an enlarged tuft between the eyes composed of long white, straight and curved scales and yellow hairs further back with long grey lanceolate* scales finally with dark grey and black upright forked scales

Thorax.—*Prothoracic lobes well developed* covered with golden brown hairs Mesonotum with a median tuft of lanceolate scales greyish in front elsewhere with numerous short golden incurved tufts Smooth paramedian lines on the anterior two-thirds of the mesonotum and lateral smooth ones on the posterior

half Very dark hairs above the wing roots Scutellum simple not trilobed yellowish grey and covered with long golden yellow hairs

Wings —First sub marginal cell short one tenth the length of the wing one third the length of its stem second posterior cell well developed about one fourth the length of the wing the stem about two thirds the length of the cell Third long vein very slightly curved inwards fifth a little more so The part of the fourth long vein which forms the stem of the second posterior cell shows an obtuse angle directed backwards There are two or three well marked pseudo veins All veins covered with dark brown spatulate scales fringe composed of straight grey pointed linear scales There are no spots the scales being of a uniform colour

Abdomen black without scales covered with golden yellow hairs more numerous on the first segment *The hairs of this segment are broader than those of* the other segments There is but one spermatheca composed of a spherical portion and a chitinous tube the length of which represents nearly two fifths the diameter of the sphere

Legs deep yellow brown without bands, ungues simple

Length —5 5 mm

The larva in life assumes the typical position of Anopheles The living larva is distinguished macroscopically by three clear zones on the dorsal surface These zones are situated on the thorax on the fourth and eighth segments of the abdomen

For descriptive purposes we adopt the nomenclature proposed by Stanton (1915)

The antennæ show a median plumose fine hair

The internal anterior clypeal hairs are situated close to the median line They *are simple The external anterior clypeal hairs are plumose* and situated on the same transverse line as the internal ones and as long as the latter The posterior clypeal hairs are small bifurcate and situated backward from the external anterior hairs

The frontal hairs are six in number and all plumose There are four occipital hairs all bifurcate and of the same length The situation of the internal pair is well behind that of the external pair arranged in a semi circle with the concavity anteriorly

The submedian thoracic hairs form two groups composed of three hairs a small simple external one one *slightly* plumose which *is the most developed* and an internal plumose one

There are two pairs of palmate hairs on the thorax none on the first abdominal segment segments 2 to 7 each have a pair The lamellæ of all these hairs are *narrowly* lanceolate and simple that is without denticles

There is no respiratory siphon

The teeth of the comb alternate irregularly one or two short teeth being placed between two long teeth this character is variable in different individuals The teeth do not show the basal fringe of spines Anal papillæ are four in number and well developed

The internal buccal organs maxillæ mandibles and mentum are seen in figures 2 4 and 5 (Brug and De Rook)

Habitat —Territory of New Guinea Muina (Biro), Beining district (Heydon and Hill) Dutch New Guinea, Pionierbivak Mamberano River (Brug and De Rook)

The material in the Institute collection a damaged male does not permit the drawing up of a description therefore those of Brug and De Rook Edwards and Theobald have been used It is a very distinct species which according to Edwards merits its retention in the genus *Bironella* instead of being merged into *Anopheles*

Brug and De Rook found the larvæ in very peaty marshes The larvæ being only slightly active and not readily disturbed were easily captured

Heydon and Hill found the larvæ in the backwash of a small mountain stream a tributary of the Nambung River in New Britain which contained a considerable quantity of decayed vegetable matter

Brug and De Rook state that though their camp was but a short distance from the breeding ground adults were never taken whereas those of *A punctulatus* var *moluccensis* were abundant

Relation to Malaria —No evidence Brug and De Rook state that it probably plays no part in the transmission of malaria

Conclusions

The life histories of the majority of the species are fairly well known though in some their variations have not been finally determined

There is yet a considerable amount to be done in connection with the distribution of the various species before their range becomes well known

It has been shown that the species of the subgenus *Anopheles* are fairly closely related and that *annulipes* and *amictus* of the subgenus *Myomyia* still require considerable research before the status of the various forms is finally established

Bironella gracilis Theob has for the present at least been retained as generically distinct from *Anopheles*

The definite relation borne by the various species to malaria has been shown to be entirely lacking except in the case of one species *A punctulatus* and its variety *moluccensis* which were proved by Heydon to be natural vectors at Rabaul The evidence concerning *A annulipes* though presumptive is not convincing inasmuch as we know that the blood of the patient had not been examined before he settled in the Sydney district (Money 1926) therefore we do not know that he was free from the malaria parasite which may have been latent in his blood even though he had not lived in a malarious country and the Sydney district cannot under any circumstances be considered such

Concerning *A amictus* and *A bancrofti* the position is rendered even more obscure since we know that *A punctulatus* and var *moluccensis* are both found in North Australia where both *amictus* and *bancrofti* are to be found in numbers

and where moreover epidemics of malaria have from time to time occurred There fore we cannot exclude the two latter species as vectors

Coming now to Queensland and North Queensland in particular we find that apparently the prevailing species is *A amictus* with a mixture of *A bancrofti* and to a much less degree if at all of *A annulipes* In addition we cannot exclude the possibility of the presence of *A punctulatus* and var *moluccensis* *Punctulatus* occurs well down the western side of the Gulf of Carpentaria as most probably also does its variety therefore it is not difficult to believe that it may be found on the Cape York Peninsula Should they so be found their presence would explain the epidemics which have occurred in the past It may be asked with reason why has malaria practically died out from Cairns for instance ? To the writer s mind the answer lies in the fact that all the important Anopheline breeding places in that locality have been filled in or destroyed in recent years

It was hoped to have illustrated this paper with drawings and photographs dealing with the adults their life histories and breeding places but time would not permit of their preparation

A map of Australia has been prepared which gives the known distribution of *Anopheles* species Where localities are close together one of them has been omitted when harbouring the same species All doubtful specific localities are likewise omitted

In conclusion I desire to tender my sincere thanks to Col J Cunningham for his kind invitation to contribute this paper to Dr J S C Elkington Director of the Division of Tropical Hygiene for permission to do so and also to my friend Dr Mackerras B Sc for the gift of specimens of *A atratipes* Skuse and *A stigmaticus* Skuse

REFERENCES

BANCROFT T L (1908) — *Ann Queensland Museum* No. 8.
BREINL A (1915) — *Ann Trop Med and Parasit* Vol IX, p 389
Idem with TAYLOR F H (1918) — *Med Jour Aust* August 10th
BRUG S L and ROOK H DE (19[illegible]) — *Bull Soc Path Exot* Vol XX No 5 p 305
CHRISTOPHERS S R (1922) — F E A T M 4th Congress Batavia 1921 p 421
Idem (1924) — *Ind Med Res Memoir* No 3
CILENTO R W (1924) — Commonwealth *Dept Health* *Service* Publ (Trop Division) No 3
COOLING L F (1921) — *Proc Roy Soc* Queensland p 166
Idem (1924*a*) — Commonwealth Dept Health Service *Publ* (Trop Division) No 1
Idem (1924*b*) — *Ibid* No 3 Appendix
COVELL, G (1927) — *Ind Med Res Memoir* No 7
EDWARDS F W (1921) — *Bull Ent Res* Vol XII p 69
Idem (1922) — *Op cit* Vol XIII p 75
Idem (1924) — *Ibid* Vol XIV p 351
FERGUSON E W (1926) — 15th Report Microbiol Lab N S W for year 1924 p 187
GILES G M (1902) — *Handb of Gnats or Mosq* Ed 2
HAMLYN HARRIS R (1927) — *Bull Ent Res* Vol XVII p 411
HEYDON G M (19 3) — *Med Jour Australia* Dec 15th

Hill, G F (1917) — Bulletin N Territory No 17
Idem. (19 9) — Commonwealth Dept Health Service Publ No 1
Idem (19 5) — *Proc Roy Soc* V ctor a 1 p 61
Kinoshita K (1906) — *Arch f Sch ffs—und Tropenhyg* Vol. X, p 714
Koidzumi M (19 4) — F F A T M 5th Congress 1923 p 86
Mackerras I M (19) — *The Aust Natural st* Vol VI pt 3 p 33.
Idem (19 7) — *Proc Linn Soc* N S W Vol LII p 33
Money E A (19 6) — *Med Journ Aust* August 28
Mapplestone P A (1923) — *Ann Trop Med and Paras t* Vol XVII p 13
Nicoll, W (1917) — *Jour Hyg* Vol XVI p 69
Stanton A T (1915) — *Bull Ent Res* Vol VI p 159
Taylor, F H (1912) — *Bull N Terr tory* No Ia p
Idem (1913) — *Rep Aust Inst Trop Med* for 1911 p 49
Idem (1914) — *Proc L nn Soc* N S W Vol XXXVIII p 747
Idem (1914) — *Trans Ent Soc* 1914 p 185
Idem (1914) — *Proc L nn Soc* N S W Vol XXXIX p 4
Idem (1915) — *Op c t* Vol XL, p 176
Idem (1916) — Commonwealth of A stralia Quarantine Serv ce Publ cation No 6 p 57
Idem (1917) — *Op c t* Serv ce Publ cat on No 1
Idem (1918) — *Proc Linn Soc* N S W Vol XLIII p
Theobald, F V (1901—1910) — Monograph of the Cul c dæ
Yamada, S (19 4) — Scient fic Reports from the Government Inst tute for Infect ous Diseases for the year 1924 V l III p 15
Idem (19 5) — *Op c t* for the year 19 Vol IV p. 447



MAP.

NOMENCLATURE DES MOUSTIQUES DE LA COCHINCHINE ET DU SUD ANNAM

PAR

E. BOREL,

Chef du Laboratoire d'Entomologie Médicale, Institut Pasteur de Saigon.

GENRE **ANOPHELES** Meigen, 1918

Sous genre ANOPHELES Christophers, 1915

Groupe ANOPHELES Root, 1922

A. aitkeni James
A. hyrcanus var. *sinensis* Wied.
A. hyrcanus var. *nigerrimus* Giles
A. barbirostris Van der Wulp

Sous genre MYZOMYIA (Blanchard) Christophers, 1915

Groupe PSEUDOMYZOMYIA [1]

Ps. subpicta Grassi
Ps. vaga Dönitz

Groupe NEOCELLIA

N. fuliginosa Giles
N. jamesi Theo.
N. maculata Theo.

Groupe NEOMYZOMYIA

N. leucosphyra Dönitz
N. kochi Dönitz
N. tesselata Theo.

GENRE **MEGARHINUS** Robineau Desvoidy, 1827

M. kempi Edwards
M. splendens Wied.
M. 68 sp. n. ?
M. 73 sp. n. ?
M. albipes Edwards

GENRE **URANOTAENIA** Arribalzaga, 1891

U lutescens Leic

U 75 sp n ?

GENRE **HARPAGOMYIA** de Meijere, 1909

H genurostris (Leic)

GENRE **TOPOMYIA** Leicester

T gracilis Leicester

GENRE **RACHIONOTOMYIA** Theobald

R proxima Edwards

R aranoïdes Theobald

GENRE **HEIZMANNIA** Ludlow

H complex Theobald

H communis Leicester

GENRE **MUCIDUS** Theobald

M laniger Wiedmann

GENRE **ARMIGERES** Theobald

Sous genre ARMIGERES Theobald

A kuchingensis var *dibrugharensis* Barraud

A moultoni Edwards

A aureolineatus Leicester

A obturbans Walker

A X sp N ?

Sous genre LLICESTERIA Theobald

L flava Leicester

L dolichocephala Leicester

L magna Theobald

L annulitarsis Leicester

L pectinata Edwards

GENRE **STEGOMYIA** Theobald

St vittata Bigot

St albopicta Skuse

St pseudalbopicta sp n ?

St albolineata Theobald

St mediopunctata Theobald
St annandalei Theobald
St indosinensis sp n ?
St edwardsi Barraud
St argentea Poiret

GENRE FINLAYA Theobald

F niveа Ludlow
F khazani Edwards
F macfarlanei Edwards
F trilineata (Leicester)
F prominens Barraud
F assamensis Theobald
F elsiae Barraud
F 89 sp n ?

GENRE AEDES Meigen

A andamanensis Edwards
A 50 sp n ?
A 51 sp n ?

GENRE ECCULEX Felt

E vexans Meigen
E caecus Theobald
E imprimens Walker
E niveoscutella Theobald

GENRE INCERTAIN

A ostentatio Leicester

GENRE TAENIORHYNCHUS Arribalzaga

Sous genre COQUILLETTIDIA Dyar

C crassipes (Van der Wulp)

Sous Genre MANSONIOIDES Theobald

M annulifera Theobald
M uniformis (Theobald)

GENRE ORTHOPODOMYIA Theobald

O anophéloides forme Albipes Giles

GENRE **LUTZIA** Theobald

L. halifaxi Theobald
L. raptor Edwards
L. fuscana Wiedmann

GENRE **CULEX** Linné

Sous Genre CULEX S. St.

C. bitæniorhynchus Giles
C. mimeticus Noe
C. mimulus Edw.
C. gelidus Theobald
C. sitiens Wiedmann
C. vishnui Theobald
C. tritæniorhynchus Giles
C. fatigans Wied.
C. fuscocephalus Theo.
C. brevipalpis Giles
C. malayi Leicester

Sous genre CULICIOMYIA Theobald

C. viridiventer Giles
C. pallidothorax Theo.

Sous Genre LOPHOCERATOMYIA Theobald

L. minutissima Theo.
L. minor Leicester
L. roubaudi sp. n. ?
L. bernardi sp. n. ?

SUR LES MOEURS DES ANOPHELES EN COCHINCHINE

PAR

F. BOREL

Chef du Laboratoire d'Entomologie Médicale, Institut Pasteur de Saigon

A. Larves

Gîtes.	Naturels	Eaux courantes	
		profon l b bords l er ux	*A s n n A f l j n m*
		Sans profonde e c u s ralenti par une v ta o abondante	*Ps nga— A In rost —A f l n a A s nens s A macu lata*
		Eaux stagnantes	
		S l-es sans ve at n aq at q e	*Ps vaga*
		Mara s ou Mar ots ave ve tat on abondante	*Ps vaga* Plus rarement *A s n n s—A maculata*
		Mares lo fa bl etend e	*Ps vaga A s nens s*
		Fl q es d ea x l coutes des p te Tr us lan ls b o fa ts p r b pieds le l hon les an u x l rones l s ba rettes	*A le co ph j a A loch*
		Vasqu s l s roch rs	*A a l n A j m s A macu lata—Ps vaga*
		Cre x d arb res	*N fuleg nosa*
	Artificiels	M r el le c n tru t o m ta ou m nt l nt ha r x re o ent les aux de plu	*Ps vag*
		l p do t u e nat n tal l q u en poter e cruq s d fr t en u a eo h rus tr e	*Ps vaga* Larem nt *A a b ros l s*
Fréquence	Influences saisonnières	T ut l'année	*I s caya A l ch A s n ns s— A fal g nosa A ma ula a*
		S on les pl A lA A l uco phyra A mi re	
		F l l so d s p l s l b rh o a n neem t h ls s son l s pl s s antes	*A l ael l A a l n A j m*

- **Frequence**
  - **Influence Solaire**
    - Vivant au soleil ou à l ombre — *Ps vaga—N kochi—A barb rostris A sinensis N tesselata N fuliginosa A aitkeni N jamesi*
    - Seulement au soleil — *N maculata*
    - Seulement à l ombre — *N leucosphyra*
  - **Influence de l altitude**
    - Region des plaines
      - Cultivées tres habitées — *Ps vaga—N kochi A barb rostris A sinensis N tesselata N fuliginosa*
      - Forêt Presque sans population — *N maculata N leucosphyra*
    - Région de Montagne — *N maculata A sinensis, N fuliginosa, A aitkeni N jamesi*

- **Ennemis Naturels**
  - Larves de culicides — MEGARHINUS—ARMIGERES—LUTZIA
  - Larves d Insectes. — Eph mères et d autres assez nombreuses non determinées
  - Batraciens
  - Poissons — Notamment dans les rizières un poisson minuscule très vorace que les indigenes appellent le con ca Thia Thia

B Adultes

- **Rapports avec les groupements humains**
  - Familiers *Ps vaga—Ps Subpicta*
  - Mi sauvages *A barb rostris—A sinensis—A nigerrimus—N kochi—N fuliginosa—N tesselata*
  - Sauvages *N maculata—N leucosphyra—A aitkeni N jamesi*

- **Déviation vers le bétail.**
  - Déviés per le bétail
    - Moustiques familiers
    - Certains moustiques mi sauvages
    - *A barbirostris*, *A sinensis*, *A nigerrimus*
  - Non déviés
    - Certains moustiques mi sauvages
    - Moustiques sauvages
    - *N kochi*, *N tesselata*, *N fuliginosa*

- **Fréquence** — Renseignements donnés dans A

- **Hibernation** — Il n y a pas à proprement parler d hibernation mais un certain repos génital peut s observer pour le plus grand nombre des especes pendant la saison sèche pour quelques autres au fort moment de la saison des pluies

- **Piqûres**
  - Toujours nocturnes
  - Rarement à l air libre

Role dans la Transmission du Paludisme

- Experiences d infestation de *Ps vaga* avec resultats n gatifs
- Pour des raisons basees sur des observations épidemiologiques
  - Especes du sous genre ANOPHELES et parmi les especes du sous genre MYZOMYIA *N tesselata* *N fuliginosa*
    - Peu suspects d un role actif Tierce bénigne avec ordinaire ment manifestations saisonnieres peu graves de beaucoup prépondérantes dans toute leur aire de dispersion Terres grises Ouest Cochinchinois
  - *N Lochi* *N leucosphyra* *N maculata*
    - Tres suspectes d'un role actif Tierce maligne de beaucoup la plus frequente, avec manifestations graves accès pernicieux mortels Tierce benigne et quarte à manifestations graves dans leur aire de dispersion Terres Rouges
- Dans un groupement humain vivant sous le meme toit l impaludation de plusieurs personnes et de ces personnes par la même espèce d hématozoaires est la regle Le paludisme parait bien etre une épidemie de maison les anopheles s habituent à venir prendre leur repas aux mêmes lieux et sans doute y demeurent pendant l epoque du repos génital

THE IDENTIFICATION AND CLASSIFICATION OF THE SPECIES OF THE GENUS *PHLEBOTOMUS* WITH SOME REMARKS ON THEIR GEOGRAPHICAL DISTRIBUTION IN RELATION TO DISEASE

BY

MAJOR J. A. SINTON, V.C., O.B.E., I.M.S.
Central Malaria Organisation, Kasauli

THE identification of the members of the genus *Phlebotomus* has been in the past a matter of the greatest difficulty, indeed it was only in the case of male specimens that any degree of certainty in diagnosis existed.

The identification of female specimens usually required very laborious and expert study and even after such study, some doubt might still exist as to the specific identity of the specimens. This was a great drawback, for it is with the females of *Phlebotomus* that the chief interest of the medical entomologist lies because the males are not known to suck blood.

Earlier Methods of Identification

The distinctive morphology of the male hypopygium made it comparatively easy in most cases to identify the males with a fair degree of accuracy. The points on which special stress was laid were the number and position of the spines on the superior claspers, the presence or absence of spines on the distal end of the inferior clasper and the presence or absence of lobulation and spines on the intermediate appendages.

Although the morphology of the male genitalia was specific for the majority of known species there were some in which no very marked differences could be found. For the final differentiation of these species reliance had to be placed upon such secondary points as size, colour, geographical distribution, variations in wing venation, palpal formulae, antennal measurements, etc. Even with these aids diagnosis was often difficult and some workers believed that when better diagnostic characters were discovered it would be found that several species had been classed together, as one, more especially in the *minutus* group.

No gross structural characters had been discovered in the female hypopygium of equal diagnostic value to those seen in the male. In the identification of specimens of this sex reliance had to be placed on secondary characters similar to those used in the male (vide *supra*). This method was so unsatisfactory that on several

specimen, indeed in areas where several very similar species are found it was sometimes impossible to be absolutely certain of the diagnosis of some specimens This was especially the case with the smaller species resembling *P minutus*

Recent Aids to Identification

An attempt was made by the author (Sinton 1925*b*) to determine whether a careful study of the minute anatomy of the female hypopygium would reveal any points of specific diagnostic importance The morphology of the furca and the number and character of the spines on the post genital ridge show features which are apparently characteristic of some species but in the majority of cases little or no help was obtained (Sinton 1927*c*)

Adler and Theodor (1926*a*) from an examination of the Palestine sandflies pointed out that in the *minutus* group the posterior portion of the floor of the buccal cavity bears a number of teeth and that there is a pigmented area of chitin at the posterior dorsal portion of this cavity The morphology of these structures seems to be specific (Sinton 1927*d*) These workers also noted that the posterior part of the pharynx is lined by a series of ridges the appearance of which as well as the shape of the pharynx they found of considerable diagnostic aid

Newstead (1911) in describing the three Maltese species of *Phlebotomus* divided them into two groups—(*a*) those in which the hairs on the dorsum of the abdomen are erect and (*b*) those in which all these hairs are recumbent except those on the first segment It has recently been noted (Sinton 1927*c*) that in the Indian species the spermathecæ of the erect haired group have a crenulated outline while in those of the recumbent haired group the outline is smooth The shape of the spermathecæ in many members of the former group seem to be specifically characteristic while in the latter group specific characters are less evident Adler and Theodor (1926*a* 1927*a*) have however described certain differences in the shape of the spermathecæ of the *minutus* group (recumbent haired species) These differences have not as yet been found of much aid in the identification of the Indian species

Several other morphological characters have been described which while sometimes of help do not appear to have the primary diagnostic character of those mentioned above Tonnoir (1921) has erected two new species—*P ariasi* and *P neglectus*—on slight differences in the morphology of the distal end of the genital filaments of sandflies of the *P perniciosus* type Pinto (1926) noted differences in the morphology of the pompetta in certain South American species This author also drew attention to variations in the shape of the distal end of the genital filaments in these species This has also been done in the case of *P squamirostris* (Sinton 1927*b*) The segmental distribution and position of the "curious sensory spines" on the palps and the number and distribution of the geniculate spines on the antenna are sometimes of diagnostic importance (Sinton 1927*b*)

When in England I was kindly allowed to study the collections of *Phlebotomus* in the British Museum and in the London and Liverpool Schools of Tropical Medicine

While doing this special attention was given to the points mentioned above It was found that the Chinese species *P perturbans* var Patton and Hindle 1926 in its buccal features and other characters seemed to be identical with *P squamirostris* Newstead from Japan (Plate IX fig 11) The buccal morphology of the Indian *P minutus* (Plate IX fig 16) differs markedly from that of the type European species (Plate IX fig 15) and it will probably have to be reinstated as *P babu* Annandale A re-examination of the type specimens will be necessary to confirm this The African species *P simillimus* Newstead is a recumbent haired' one while the Indian one *P simillimus* var *hospitii* Sinton has erect hairs and so becomes a separate species as *P hospitii* *P minutus* var *montanus* has a very markedly different buccal morphology (Plate IX fig 17) to either *P minutus* or *P babu* and is evidently a separate species *P montanus* as had been suspected for some time *P christophersi* Sinton an erect haired species has also been separated from *P minutus* with which it has previously been confused *An examination of P major along these lines has shown that under* this name have been included two distinct species with markedly different spermathecæ (Plate IX figs 3 to 5)* These species also show minor but distinct differences in the ridges of the pharynx as well as in the position of the sensory spines on the 3rd palpal segment although the male genitalia appear almost identical One of these species seems to be identical with *P perniciosus* and the other to be closely allied to *P major* var *chinensis* A re-examination of the type specimens of *P major* Annandale will be necessary to determine the synonymy of these species

These are some instances in which the methods of identification described above have proved of value It is hoped that when all species have been examined along these lines most of the present difficulties in synonymy may be cleared up †

The Classification of the Genus Phlebotomus

França (1919) attempted to classify the genus *Phlebotomus* by dividing it into two subgenera on the characters of the male genitalia Eventually this worker in conjunction with Parrot (França and Parrot 1921) created five subgenera namely *Prophlebotomus* *Phlebotomus* s restr *Brumptomyia* *Lutzia* (emend *Lutzomyia* França 1924) and *Sergentomyia* These subgenera were divided as follows —

1 The anterior branch of the 2nd fork of the 2nd *longitudinal vein of the* wing is shorter or hardly as long as the distance between the forks of this vein The inferior clasper is unarmed *Prophlebotomus*

2 The anterior branch of the 2nd fork of the 2nd vein is longer than the distance between the forks of the vein Inferior clasper armed or unarmed

---

* More recent work (Sinton 1928) has shown that one of these species is *P major* (Plate IX fig 3) while the other is *P chinensis* (Plate IX figs 4 and 5)

† Working on these lines the synonymy of most of the Asiatic species has been settled (Sinton 1928)

(*A*) Distal segment of the superior clasper of the male genitalia equal or nearly equal to the length of the proximal segment

(i) Intermediate appendage complex inferior clasper armed *Phlebotomus* s restr

(ii) Intermediate appendage simple inferior clasper unarmed *Brumptomyia*

(*B*) Distal segment of the superior clasper much shorter than the proximal one

(i) Intermediate appendage armed *Lutzomyia*

(ii) Intermediate appendage unarmed *Sergentomyia*

The Asian species would be divided as follows according to this classification —

Prophlebotomus —*perturbans christophersi palestinensis shortti africanus minutus* and its varieties, *squamipleuris* (?) *squamirostris* (?)

Phlebotomus s restr —*papatasii*

Lutzomyia —*argentipes newsteadi*

Sergentomyia —*himalayensis malabaricus chalami montanus nicnic squamipleuris* (?) *squamirostris* (?) *hospitii major perniciosus sergenti* lt *crinaceus colabaensis*

This classification seems to me to be very unsatisfactory for species which appear to be closely allied such as *P minutus* and the African species *P simillimus P perturbans* and *P chalami* fall into different subgenera while the subgenus *Sergentomyia* includes a large number of very dissimilar species The main division is based on wing venation which is in my opinion not a feature of primary diagnostic value in this genus Some species such as *P squamipleuris* and *P squamirostris* might be placed in either of two subgenera according to the specimen examined and the personal inclination of the worker

It is suggested that it would be much more natural to divide the genus into three main groups —(*A*) an 'erect-haired' group with crenulated spermathecæ (*B*) a 'recumbent haired' group with smooth spermathecæ and (*C*) a group containing *P squamipleuris* (Sinton 1927*c*)

(*A*) 'Erect haired' Group

The members of this group have some hairs on the dorsum of the abdomen erect in addition to those on the first segment which are erect in all the described species of *Phlebotomus* This group is also characterized by the fact that the outline of the spermathecæ is crenulated

Two main subdivisions are found in this group —

Subdivision (*a*) Here the erect abdominal hairs are numerous usually forming tufts and present on most segments the buccal armature and pigmented area are absent or very rudimentary the spermathecæ and the male genitalia have usually a specific morphology The morphology of the pharynx may act as a subsidiary aid in diagnosis The Indian species which have so far been placed in this group are —*P papatasii P argentipes P newsteadi P major* (both types) *P sergenti* and *P colabaensis* (Plate IX figs 1 to 7)

Sub division (*b*) The species in this sub division have scanty erect hairs on the abdominal segments especially in the male, the buccal armature and pigmented area are present and have a specific morphology (Plate IX figs 13 and 14) The morphology of the spermathecæ and the male genitalia have not so markedly specific a morphology as in the previous sub division (Plate IX, fig 8) The following Indian species belong to this group *P hospitii* and *P christophersi*

(*B*) Recumbent haired' Group

The dorsal abdominal hairs except those on the first segment are all markedly recumbent and the spermathecæ have a smooth outline (Plate IX, figs 9 to 11) The buccal armature and pigmented area are well developed and up to the present all species examined have shown a distinctive morphology (Plate IX figs 15 to 17) The male genitalia have in some species a distinctive morphology, but this group includes a number of species with the *minutus* type of genitalia In the identification of these species one has to depend on other features for the final diagnosis It is possible that these should be separated off as a sub division of the group *P squamirostris* shows a few faint crenulations near the head of the spermatheca (Plate IX fig 11)

The spermathecæ in the recumbent haired' group are all more or less ovoid in shape and are much less heavily chitinized than in the 'erect haired' group For this reason they are more liable to distortion in balsam specimens and have not so far been found to show features of primary diagnostic importance in the classification of Indian sandflies It is in species belonging to this group that Adler and Theodor (1926*a* 1927*a*) find the morphology of the pharynx of value in diagnosis

The following species found in India belong to this group —*P himalayensis* *P minutus* and its varieties *P babu* *P montanus* *P malabaricus* *P shortti* *P perturbans* *P chalami*

(*C*) The Intermediate or *Squamipleuris* Group

The only species known to belong to this group is *P squamipleuris* in which some specimens show a few erect hairs on the dorsum of the abdomen while in others no traces of such hairs can be found The spermathecæ of this group are turnip shaped and highly chitinized, they are not crenulated but show a series of transverse rows of small spines (Plate IX fig 12) This species differs from all the other described species in the presence of tufts of flat mosquito like scales on the thoracic pleuræ, in the unilateral geniculate spines on the antenna of the female in the absence of geniculate spines on the IIIrd antennal segment and in the presence of sensory spines on both the 2nd and 3rd palpal segments These features seem to separate this species very markedly from the other two groups and make it worthy of a separate one The buccal armature and pigmented area of this species appear to be characteristic (Plate IX fig 18)

It is not suggested that these groups have been studied in sufficient detail to form them into subgenera, but that they are an attempt to obtain a natural

classification of the genus *Phlebotomus* This has already been found to facilitate greatly the identification of species It should aid very materially in the compilation of identification tables especially for the female sex No table has ever been attempted for this sex, in which were included more than a few species This was due to the impossibility of doing so with the unsatisfactory methods of identification available in the past

Suggestions for the description of New Species

The absence of any standard method for the description of species of *Phlebotomus* has given rise in the past to many very inadequate descriptions and to much confusion In order to overcome this difficulty and produce a uniform method França and Parrot (1920) suggested that the description of all species should be accompanied by drawings of those parts of the insect which had a distinctive morphology and that a phlebometric table should be compiled for each species Such tables contain the measurements of all the important parts of the body and its appendages and also give the relative proportions of certain structures For ease of description and record these workers suggested that certain terms and symbols should be used to denote certain parts of the wing venation and the relative lengths of other structures (*vide* Sinton 1923) This method was a distinct advance and has done much to systematize the description of sandflies in recent years

Accurate descriptions and drawings of the structures which have recently been found of diagnostic value will probably do away with the necessity for much of this laborious method of measurement It is my opinion however that until the more recent methods have been fully tested on specimens from all parts of the world the phlebometric method should also be used in all descriptions of new species An attempt is being made to describe all the known Indian species along these lines

THE GEOGRAPHICAL DISTRIBUTION OF *Phlebotomus* IN RELATION TO DISEASE

The only disease which has been definitely proved to be carried by sandflies is the fever known as sandfly or *Phlebotomus* fever Townsend (1927) claims that he has transmitted the South American disease verruga by the bite of *P verrucarum* but this awaits confirmation Very strong evidence however has been brought forward in recent years that leishmaniasis both visceral and cutaneous is carried by these insects

Our knowledge of the geographical distribution of the different species of *Phlebotomus* is still in its infancy This is accounted for by the difficulties which existed in specific identification of these insects A comprehensive survey of any area is a tedious and often a lengthy task on account of the limited seasonal prevalence and very localized distribution of many species

Sandfly Fever

Up to the present the only species of *Phlebotomus* which has been definitely proved to be a carrier of this disease is *P papatasii* Various workers have brought forward evidence chiefly on epidemiological grounds that *P minutus* var *africanus* *P perniciosus* *P neglectus* *P major* var *chinensis* and *P sergenti* may also act as carriers

The known distribution of this disease in India at least is very similar to that of *P papatasii* but outbreaks of a disease with somewhat similar clinical characters have been reported from areas where this species is not known to exist This discrepancy may depend upon (*a*) the presence of another vector either another species of *Phlebotomus* or some other insect, (*b*) insufficient knowledge of the distribution of *P papatasii* or (*c*) mistaken diagnosis of the disease which can at present only be identified by clinical symptoms

It is evident that we require further work on the transmission of the disease by species of *Phlebotomus* other than *P papatasii* a more accurate method of diagnosis of the disease and further work on the distribution of the different species of *Phlebotomus*

Leishmaniasis

The evidence is very strong that leishmaniasis is carried by some species of *Phlebotomus* The only link missing in the chain appears to be the experimental transmission from the fly to man under conditions which are likely to occur in nature

Visceral Leishmaniasis The observation made in 1922 (Sinton 1924 1925*a*) that the known distribution of kala azar in India corresponds closely with that of *P argentipes* led Knowles, Napier and Smith (1924) to carry out their experiments with this species They then found that specimens of *P argentipes* which had been fed on cases of kala azar showed a development of *L donovani* in their gut Since that time this observation has been confirmed on other occasions by different workers especially the Indian Kala azar Commission

I believe that this species has been found in every endemic area of Indian kala azar where a systematic search has been made by competent observers The species has however been recorded from places where the disease is not known to occur Although this discrepancy may be accounted for by assuming that *P argentipes* is not the vector of kala azar it may also be due to a condition like the presence of known malaria carrying Anophelines without malaria or that the disease has escaped notice or that the meteorological conditions are unfavourable for the development of the parasite in the fly

It is interesting to note that since Savage (1927) reported a case of apparently indigenous kala azar at Sanawar in the Simla Hills at a height of about 5 000 feet a few specimens of *P argentipes* have been collected from a neighbouring village This is a unique record, for neither this species nor kala azar had previously been recorded at heights above 2 000 feet nor so far west in India

Cutaneous Leishmaniasis Sergent (Ed and Et) Parrot Donatein and Beguet (1921) produced a cutaneous leishmaniasis in man by the inoculation of crushed sandflies caught in nature and identified as *P papatasii*

Adler and Theodor (1925 1926*b* 1927*b*) and Parrot and Donatein (1926) have produced this disease in man by the inoculation of specimens of *P papatasii* which had been found either infected in nature or had been infected by feeding on sores or cultures of *L tropica* In no case have they reported successful results in transmitting the disease by the bites of these insects

Adler and Theodor (1927*b*) only managed to produce an infection in 5 6 per cent of *P papatasii* fed on human cases of oriental sore which is a smaller percentage than the 22 5 per cent of infected *P argentipes* found by Christophers Shortt and Barraud (1926) after feeding on kala azar cases From this it would seem possible that if *P papatasii* is a carrier of cutaneous leishmaniasis it is not likely to be such a suitable one as *P argentipes* may be in kala azar

P papatasii has a regional distribution which is much wider than the known distribution of oriental sore It was pointed out (Sinton 1922) that of all the recognized species of *Phlebotomus* the known distribution of *P sergenti* was the one which most closely corresponded with that of this disease Further records were collected and of these twenty two were from known and in most cases notorious foci of this disease two were from areas where the disease probably occurs and two were from places where the disease is said not to exist (Sinton 1925 1927) Since that time specimens have been examined by me from China and Aleppo and also from Karnal and Sulkur in India The four latter places are well known as centres of oriental sore but no records are available from China It is of interest that the specimen from Aleppo was found amongst the collection made by Wenyon (1912) when he first recorded the presence of herpetomonads in *Phlebotomus* Up to the present *P sergenti* has been recorded from eight different places in India all of which are known centres of cutaneous leishmaniasis

The distribution of *P sergenti* in relation to that of oriental sore is very suggestive and it seems that experimental work with this species might repay further study

Conclusion

Our present knowledge of the geographical distribution of the different species of *Phlebotomus* is very inadequate and the increasing importance of this genus in medical entomology clearly indicates that the subject is worthy of a more complete study It is hoped that since the specific identification of the members of this genus has become less difficult and more accurate other workers will interest themselves in the subject

References

Adler and Theodor (1925) *Nature* Vol CXVI No 2913 pp. 314 315

Idem (1926*a*) *Bull Ent Res* Vol XVI No 4 pp 399—405

Idem (19 6/)	*Ann Trop Med anl Iaras t* Vol XX No ° pp 175—194
Idem (19°7a)	*Ib d* Vol XXI No 1 pp 61—69
ADLER and THEODAR (19°7b)	*Ibid* Vol XXI No ° pp 89—101
CHRISTOPHERS SI ETT and BARRAUD (19 6)	*Ind Med Res Memo rs* No 4 pp 141—145
FRANCA (1919)	*Broteria (Ser Zool)* Vol. XVII 2/3 pp 147 148
Idem (19 4)	*Jour de Sci Nat F s c et Nat* (Ser 3) No 17 pp 10 11
FRANCA and PARROT (19 0)	*Bull Soc Path Fxot* Vol XII No 8 pp 695— 08
Idem (19°1)	*Arch Inst Past Afr Nord* Vol I No 3 pp °79—°84
KNOWLES NAPIER and SMITH (19 4)	*Ind Med Ga* No 1° pp 593—597
NEWSTEAD (1911)	*Bull Ent Res* Vol II p 68
PARROT and DONATEIN (19°6)	*Bull Soc Path Exot* Vol. XIX No 8 pp 694—696
PATTON and HINDLE (19 6)	*Proc Roy Soc* C B 05 pp 405—41°
PINTO (19°6)	*Sc encia Mel* Vol. IV No 7 pp 3°0—375
SAVAGE (19 7)	*Ind Med Ga* No 6 pp 38°—384
SERGENT SERGENT PARROT DONATEIN and BROUET (19 1)	*C R Hebd Acad Sc* Vol CLXXIII No 21 pp 1030—103
SINTON (19°°)	*Ind Jour Med Res* Vol IX No 3 p 579
Idem (19 3)	*Ib d* Vol X No 3 pp 74°—744
Idem (19 4)	*Il d* Vol XI No 4 pp 1035—1049
Idem (19°5a)	*Ibid* Vol XII No 4 pp °10—7°7
Idem (19 5b)	*Ib d* Vol XIII No 1 pp 97—107
Idem (19°7a)	*Ib d* Vol XIV No 4 pp 941—945
Idem (19° b)	*Ib d* Vol XIV No 4 pp 947—953
Idem (19°7c)	*Ib d* Vol XV No 1 pp 21—°
Idem (19°7d)	*Ib d* Vol XV No 1 pp °9—3
Idem (19°8)	*Ibid* Vol XVI No pp °97—3 4
TONNOIR (19 1)	*Ann Soc Ent m Belg* Vol LXI pp 53—56 pp 333—335
TOWNSEND (19 7)	*In Rev Appl Entom* Vol XV B 9 pp 159 160
WENYON (191°)	*Jour London S h Trop Med* Vol I No ° p 98

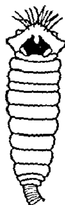


Fig. 1.



Fig. 2.

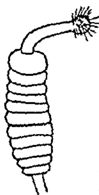


Fig. 3.



Fig. 4.



Fig. 7.



Fig. 8



Fig. 9



Fig. 11.

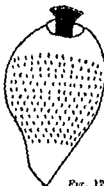


Fig. 12.

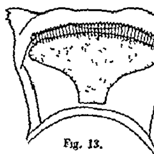


Fig. 13.







Explanation of Plate IX

Fig 1 Spermatheca of *P papatisu*
2 *P arge t pes*
3 *P i ajor*
4 *P major var cl i e sis* (In l a)
5 *P major var cl ine isis* (China)
6 *P custeadi*
7 *P sergenti*
8 *P christoj l ersi*
9 *P i tus var ante atus*
10 *I i o ta us*
11 *P sq a i rostr s*
12 *I squa i pl uris*
13 Buc al armature of *I lo p ti*
14 *I chr topl ers*
15 *P i tus var te t i*
16 *P tus (I babi)*
17 *I u o ta i is*
18 *P sq amipleuris*

THE BREEDING OF SANDFLIES IN NATURE AND IN THE LABORATORY

BY

R O A SMITH, D T M, I M D

THE well founded suspicion that sandflies (*Phlebotomus*) may be vectors of dangerous diseases like kala azar in addition to simple ones like sandfly fever and oriental sore has drawn attention to these flies in various parts of the world where these diseases are prevalent and the study of the bionomics of the genus has increased in importance

The following is an account of the various methods used in the search for breeding places in nature and in breeding the flies in the laboratory

Breeding in Nature

The main requirements for breeding of sandflies are the presence of a certain degree of moisture absence of sunlight suitable conditions of temperature and the presence of any form of decomposing nitrogenous matter on which the larvæ can feed

The reports of occasions when *Phlebotomus* larvæ have been found breeding in nature are remarkably small considering the prevalence of this genus and its importance in medical entomology Their small size and colour, and their preference for dark corners have increased the difficulty of finding them

Larvæ of *P minutus* have been found breeding in nature by Howlett in Pusa and Mitter in Lahore Larvæ of *P papatasii* were found by Mitter in Lahore and McCombie Young Richmond and Brendish in the Peshawar district The larvæ of *P argentipes* have been found by the author in Calcutta and Raman in Madras

Newstead Grassi and Marett have found larvæ in Malta and King in the Soudan

Sandflies have been found breeding in a variety of situations—in such highly manured soil as that from a stable where cows and goats were housed as well as in the dust from cracks in a cement floor where the only food available for the larvæ was the remains of cockroaches and other such insects

The cycle from egg to adult takes about 30 days in summer and two to three months in winter

In the search for larvæ the correct time of the year when the temperature conditions are most suitable for breeding, should be chosen and the vicinity of houses and stables where flies are found should be examined within a radius of 50 yards Samples of material should be collected from likely spots and examined for the presence of larvæ

The suspected material may be examined directly with a hand lens This method is only possible when the sample is a small one—when the amount of material is large it may be placed in a suitably covered vessel and all emerging Diptera examined The method which has yielded the best results is that described by McCombie Young Richmond and Brendish The suspected material was washed through three sieves of different sized mesh and the material held up by the finest-meshed sieve was placed in a vessel containing some water and allowed to sediment The floating debris was thoroughly agitated to dislodge any larvæ which might have been adhering to it and then skimmed off Any excess of water was syphoned away and the residue transferred to a large enamel photographic dish To the residue was added a saturated solution of granulated sugar The alteration in the specific gravity of fluid caused all larvæ present to float to the surface from whence they were easily collected and examined

Large numbers of larvæ were found on many occasions by the use of this method

Larvæ migrate freely in search of food and of suitable conditions of moisture and temperature and in a uniform sample the number of larvæ found depends on the amount of material collected

Pupæ have been found in the same samples with larvæ of various sizes showing that successive batches of ova have been deposited in a given spot from time to time The pupæ are usually found in the upper layers of the material in which the larvæ are feeding so that the emerging flies can easily free themselves and move out in search of food

Breeding in the Laboratory

To breed sandflies in the laboratory for any experimental work it is advisable to confine single females in a suitable receptacle collect the ova and hatch these from each fly separately *P argentipes* bred in the laboratory oviposit readily in a test tube if kept at the correct temperature and given the requisite degree of moisture Wild flies do not oviposit so readily and do better in a larger receptacle so that a large lamp chimney suitably arranged over damp filter paper or plaster of Paris has been found most efficient

The filter paper or plaster of Paris block on which the ova are laid may be placed in a suitable vessel and kept damp when the larvæ will hatch out *in situ* But the best results have been obtained by the following method

The ova are washed or brushed into a small earthenware pot lined with a thin layer of plaster of Paris some food is placed in the spaces between the ova and the pot, covered with a piece of muslin to protect it from other flies as well as from

ants and mites is placed in a tray containing wet sand or damp cotton wool The pot is then incubated at the temperature found most suitable for the flies under experiment

P argentipes breed best at a temperature of 28° C and *P minutus squamipleuris* and *papatasii* too have been successfully bred out at this temperature

Pots containing ova should be allowed a fair degree of moisture—they should feel damp to the touch but there should be no free water The young larvæ require less moisture than the ova and as they grow the amount of moisture necessary decreases till during pupation practically no moisture is required

The most convenient food for the larvæ in a laboratory is the crushed fæces of rabbits or goats mixed with a small quantity of dried blood The pots should be examined from day to day and the amount of food and moisture regulated

The pots containing pupæ are best placed in a separate breeding out cage from which the flies may be caught and used as required

By the use of this method numerous broods of *P argentipes papatasii minutus* and *squamipleuris* have been successfully bred out

For *P papatasii* a method which has given better results is that described by McCombie Young and his collaborators A small earthenware pot is half filled with dry mule litter and a thick layer of mud put over the litter A crucial incision is made in the mud and the pot placed out in the sun so that the mud hardens and further cracks form in it When the mud is thoroughly sun baked it is stood in a Petri dish of water and the dish and pot placed in a muslin cage with some fed females of *P papatasii* The flies enter through the cracks and oviposit in the deeper layers of the litter the larvæ hatch out *in situ* and develop uninterruptedly The full grown larvæ usually come to the surface of the mud and exposed sides of the pot to pupate

This method has been used by the author in Calcutta with very good results

Receptacles in which sandflies are breeding should be protected from a species of mite belonging to the family Gamasidæ These mites destroy the ova and young larvæ

Ants too destroy the larvæ Certain other rapidly developing larvæ like those of *Mycetophilidæ* by feeding on all the available food material starve out the sandfly larvæ

In nature a certain proportion of sandflies are found infected with gregarines *Monocystis mackiei* (Shortt and Swaminath 1927) When breeding in the laboratory a large proportion of flies show these gregarines but they do not seem to influence the life history of the fly however heavy the infection may be On the other hand a species of nematode which has been found infecting *P argentipes* causes the death of the fly during either the first or second oviposition The larvæ of this nematode which are hatched in the cœlomic cavity of the fly infest the ovaries and possibly by blocking the oviduct prevent oviposition and thus cause its death

Such flies can generally be recognized and should be discarded to prevent the spread of the infection to the next generation if the flies are being bred for experimental purposes

DISCUSSION

Lieut Col T C McCombie Young I M S (B India) I would like to ask Dr Smith if he has experimented with the breeding of *P argentipes* by the earth crack method We arrived at the method as a test of breeding grounds from which it appears that *P papatasii* chooses an earth crack for oviposition

It would be interesting to know if *P argentipes* also does well by this method as, if not it would rather indicate that their breeding grounds are not in earth cracks

Dr R O A Smith I M D (Assam) replied *P argentipes* have not been found to breed as well as *P papatasii* in the pots prepared by the method of McCombie Young and his collaborators for *P papatasii* Larvæ of *P argentipes* found in nature have been found in situations of a different nature from those in which *P papatasii* were found breeding They have almost always been found inside the houses in cracks in the cement floors rat holes etc

STUDY ON THE SEASONAL PREVALENCE OF HOUSE FLIES IN CHOSEN (KOREA)

BY

HARUJIRO KOBAYASHI,

Keijo Imperial University, Medical Faculty, Keijo, Chosen, Japan

CONTENTS

INTRODUCTION
EXPERIMENTS IN THE LABORATORY
- *Method of Feeding*
- Results
- Length of Life
- Oviposition

COLLECTION OF FLIES FROM HOUSES
- Materials
- Discussion
  - *A* *Musca domestica*
  - *B* Other House occurring Flies
- Overwintering of Flies

METEOROLOGICAL CONSIDERATIONS
CONCLUSIONS

INTRODUCTION

DURING the past twenty years I have been engaged on a study of the house occurring flies from the medical point of view Special effort has been given to the problem since 1916, when I came to my new position in Cho sen (Korea) where there is known to be an abundance of flies Most parts of the study are not yet completed The following reports have been published by me dealing with certain phases of the subject —

1 1908 On the Habit and the Development of *Musca domestica* (Jap) *Dobutsugaku zasshi (Zool Magazine)* No 250
2 1913 Studies on Flies I (Jap) *Saikingaku zasshi (Journal of Bact)* No 208
3 1914 Ditto II (Jap) *Ibid* No 222
4 1918 Flies in Chosen I (Jap) *Chosen Igakkai zasshi (Journal of Chosen Medical Assoc)* No 24
5 1918 House Frequenting Flies and Their Seasonal Prevalence in Japan and Korea *Mitt d Medic Fachschule z Keijo* II

6 1921 Flies in Chosen, II (Jap) *Chosen Igakkai zasshi* No 34
7 1921 Overwintering of Flies *Jap Med World* I 3
8 1922 Flies in Chosen, III (Jap) *Chosen Igakkai zasshi* No 38
9 1922 Further Notes on the Overwintering of House Flies *Jap Med World* II 7
10 1924 On the Habits of House Frequenting Flies in Korea *Mitt d Med'c Hochschule z Keijo* VII 4
11 1925 Causes of the Abundance of Flies in Chosen (Jap) *Mansen no Ikai (Med World in Chosen and Manchuria)* No 48
12 1925 On the Propagation of Flies (Jap) *Tokio Ijishinshi (Tokio Med News)* No 2419

The tenth report in the above list dealt especially with the seasonal prevalence of the house frequenting flies summarizing the results of researches carried on during 1916 to 1923 The present communication is the sum of these studies adding experiments and observations made after the above reports were published Many data are those from Keijo (Seoul) while some of them are from several other localities in Chosen

My researches comprise three parts (*A*) Experiments in laboratory feeding the adult flies and the larvae as a research on the capacity for reproduction (*B*) Collections of house occurring flies in natural haunts to determine the seasonal prevalence of flies (*C*) Meteorological observations

EXPERIMENTS IN THE LABORATORY

Method of Feeding To determine the reproduction capacity of flies adult flies and the larvae were fed in some container After experimenting with several things, I have adopted the broad test tube as a feeding apparatus The tube has certain defects for the purpose still it affords many conveniences by its small size and easy handling

For adult flies a broad test tube of about 230 × 45 mm size was used About ten flies were put into the tube the opening being plugged loosely with cotton The tube was laid transversely on the table One piece of biscuit was put in the tube and a few drops of peptone water (5 per cent) were poured on this biscuit daily * The amount of the peptone water is an important point in the feeding

When the air in the laboratory is dry about 1 c c of fluid is necessary while in rainy season one drop of it is enough Too much fluid results in a high percentage of humidity of the air in the tube which seems to be very harmful for the life of

* In the early feeding experiments pure water was used instead of peptone water The flies under experiment survived for a long time but the ovaries of the female flies grew very slowly and the oviposition occurred very seldom Then animal blood was used instead of the water and the ovaries matured very soon and I have got many hatchable eggs Later peptone water was used for the purpose getting similar results to that with the blood After this experience the last mentioned nutriment has been used in the experiments

flies Too little amount of water results often in the death of flies from thirst The amount must be regulated according to the relative humidity of the laboratory Experience has taught me that the biscuit should be completely dry before giving the fluid then the flies are thirsty and swarm on the biscuit as soon as the fluid is given Moulded biscuit must be replaced by fresh When the wall of tube is spoiled by the excrement of flies the tube should be replaced by a new one In short the whole apparatus must be kept clean

The date and frequency of copulation oviposition and death of flies under experiments were noted daily

When oviposition was noted the flies in the tube were transferred to another tube After the hatching of maggots ' okara * was put into the tube as food for the maggots For the larvæ of *Musca domestica Muscina stabulans Fannia canicularis* and *Stomoxys calcitrans* the ' okara ' is a very satisfactory food For the carnivorous flies *Sarcophaga Calliphora* and *Lucilia* it was necessary to add some animal matter to the okara Mature maggots were transferred to another tube containing a mass of cotton in which the maggots pupate The date of pupation and emergence of adult flies were noted daily also Thus the flies lived and propagated in the tubes for their whole generation

Results The main results in experiments with *Musca domestica* can be summarized in the following paragraph

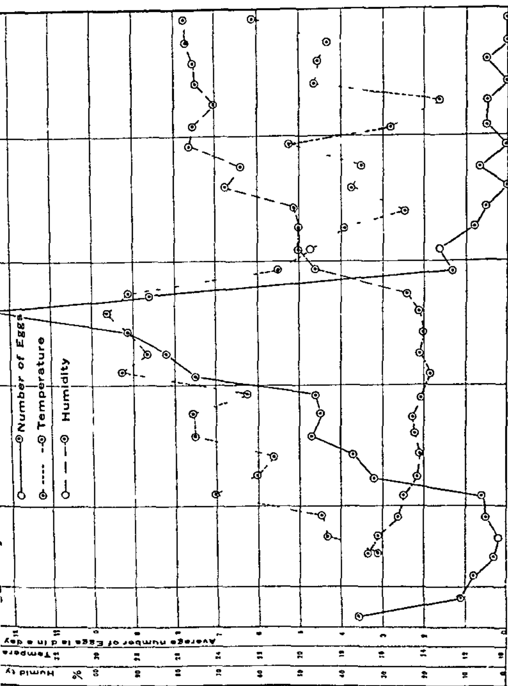
Length of life In winter about 2 to 5 months In summer about one month In other seasons the length of life is intermediate to above extremes

Oviposition *Musca domestica* and other house occurring flies have a pre oviposition period that is a newly emerged fly has an immature gonad and certain days are necessary for the first oviposition after its emergence the shortest time being three days in my experiments Further flies oviposit repeatedly

The pre oviposition period and the intervals between ovipositions are variable according to the conditions afforded to the flies, i e the relative humidity the temperature the quality of food Under unfavourable conditions the period and the intervals are prolonged and in certain cases oviposition does not occur at all The influence of food is mentioned above As to the temperature it is necessary for it to be higher than 15° C and a very favourable degree is about 30°C The relative humidity is a very important factor on the life of flies Everyone knows that flies are inactive on a rainy day and very active under the sunlight The flies which were fed in the tube oviposit very frequently in winter when heating of the room is carried on regularly while in other seasons it is less frequent I will show the result of feeding of *Musca domestica* from January to May in this year (Chart I) In the chart the average number of eggs laid

---

* kind of
alt
the


Number of Eggs
Temperature
Humidity
Average number of Eggs laid in a day
Temperature
Humidity %

in a day* the average temperature and the average relative humidity† in the laboratory are shown Each figure denotes five days average

The chart shows the frequent oviposition in the hot dry periods and its infrequency *during the lower temperature and higher humidity* periods

Similar experiments were carried on during two previous years and with similar results Unfortunately I have no exact data on the temperature and the humidity so that I will omit here any description of these experiments

Upon *Sarcophaga irrequtata* and *Calliphora erythrocephala* I got similar results to those with *Musca domestica* In *Muscina stabulans Fannia canicularis* and *Lucilia sericata* copulation took place very seldom in the enclosure and I got few fertilized eggs Further the oviposition is retarded when copulation does not occur *In the present communication I will omit all details of these studies*

Collections of Flies from Houses

Materials For the purpose of getting data on the seasonal prevalence of flies I have collected the house occurring adult flies every day during the whole year The methods of collection are —(*A*) collection by clockwork apparatus (*B*) collecting comatose flies in certain rooms after fumigation with pyrethrum powder (*C*) placing tanglefoot paper in a definite place (*D*) collection with fly flaps effort being paid to collect as many as possible (*A*) (*B*) and (*C*) were carried out in a house in which the flies were abundant while (*D*) was used in a house in which the flies were less numerous The above methods have each some defects in results Still all methods seem to give fairly good results in a broad sense when they were carried out with care Collected flies were counted separately according to the species and each five days average was figured on the charts In the 10th paper of the above list of my papers the results obtained from 1916 to 1923 in Keijo are reported and discussed In the present paper the results in Keijo from 1924 to 1926 and in Fusan for 1926 and 1927 are given in the Charts II to VII ‡

Discussion From the above charts and those of the former report we can summarize broadly in the following manner, neglecting some special cases

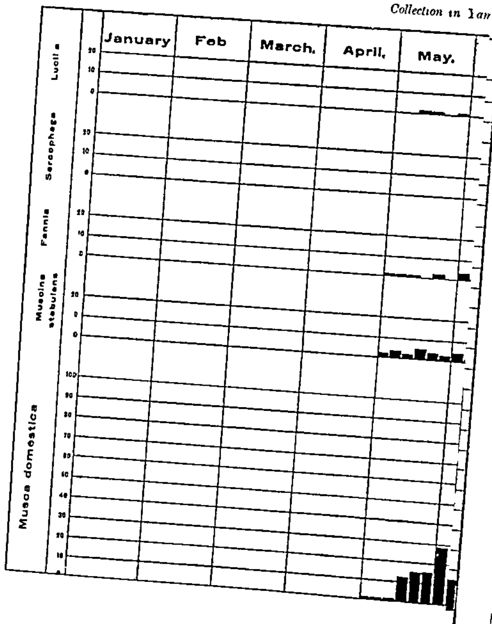
(*A*) *Musca domestica* This species comprises the main part of the collection and the seasonal prevalence of this species shows that of all flies in houses

Flies are present the whole year (*refer* also to the paragraph regarding to the overwintering) It is not rare in March and April During May and June they reach the highest numbers In the mid summer they diminish more or less remarkably In the early autumn they increase again They diminish gradually

* The number of eggs signifies the number of all eggs laid each day divided by the number of female flies for the same day About 200 to 400 female flies were fed in 80 tubes during the experiment

† Naturally the relative humidity in the laboratory must be lower than that of the tube I regret to say that I had no chance to observe directly the humidity in the tube It can be safely said however that the humidity in the *tube is proportionate to that of the exterior that* is the laboratory As is mentioned above the necessary amount of fluid varies more than 10 times according to the dry or wet weather

‡ Shading means the interruption of collection


January
Feb
March.
April.
May.
Lucilia
Sarcophaga
Fannia
Muscina stabulans
Musca domestica

in a day* the average temperature and the average relative humidity† in the laboratory are shown Each figure denotes five days average

The chart shows the frequent oviposition in the hot dry periods and its infrequency during the lower temperature and higher humidity periods

Similar experiments were carried on during two previous years and with similar results Unfortunately I have no exact data on the temperature and the humidity so that I will omit here any description of these experiments

Upon *Sarcophaga irregulata* and *Calliphora erythrocephala* I got similar results to those with *Musca domestica* In *Muscina stabulans* *Fannia canicularis* and *Lucilia sericata* copulation took place very seldom in the enclosure and I got few fertilized eggs Further the oviposition is retarded when copulation does not occur In the present communication I will omit all details of these studies

Collections of Flies from Houses

Materials For the purpose of getting data on the seasonal prevalence of flies I have collected the house occurring adult flies every day during the whole year The methods of collection are —(*A*) collection by clockwork apparatus (*B*) collecting comatose flies in certain rooms after fumigation with pyrethrum powder (*C*) placing tanglefoot paper in a definite place (*D*) collection with fly flaps effort being paid to collect as many as possible (*A*) (*B*) and (*C*) were carried out in a house in which the flies were abundant while (*D*) was used in a house in which the flies were less numerous The above methods have each some defects in results Still all methods seem to give fairly good results in a broad sense when they were carried out with care Collected flies were counted separately according to the species and each five days average was figured on the charts In the 10th paper of the above list of my papers the results obtained from 1916 to 1923 in Keijo are reported and discussed In the present paper the results in Keijo from 1924 to 1926 and in Fusan for 1926 and 1927 are given in the Charts II to VII ‡

Discussion From the above charts and those of the former report we can summarize broadly in the following manner, neglecting some special cases

(*A*) *Musca domestica* This species comprises the main part of the collection and the seasonal prevalence of this species shows that of all flies in houses

Flies are present the whole year (*refer* also to the paragraph *regarding to* the overwintering) It is not rare in March and April During May and June they reach the highest numbers In the mid summer they diminish more or less remarkably In the early autumn they increase again They diminish gradually

---

* The number of eggs signifies the number of all eggs laid each day divided by the number of female flies for the same day About 200 to 100 female flies were fed in 80 tubes during the experiment

† Naturally the relative humidity in the laboratory must be lower than that of the tube I regret to say that I had no chance to observe directly the humidity in the tube It can be safely said, however that the humidity in the tube is proportionate to that of the exterior that is the laboratory As is mentioned above the necessary amount of fluid varies more than 10 times according to the dry or wet weather

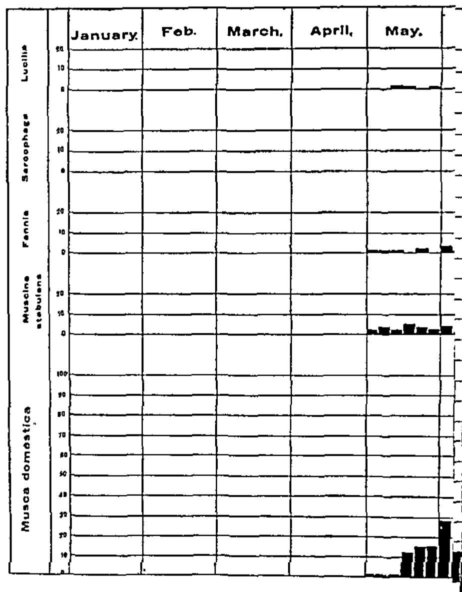
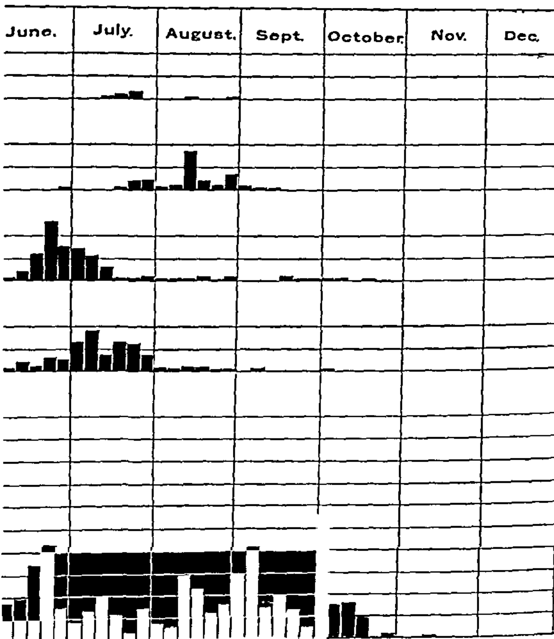

January.
Feb.
March.
April.
May.
Lucilia
Sarcophaga
Fannia
Muscina stabulans
Musca domestica
20
10
0
100
90
80
70
60
50
40
30
20
10

Chart III.

ato-Cho, Keijo, during 1924.



Collection in

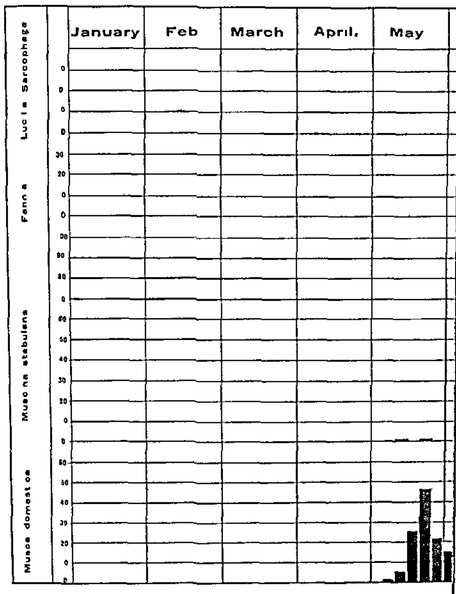
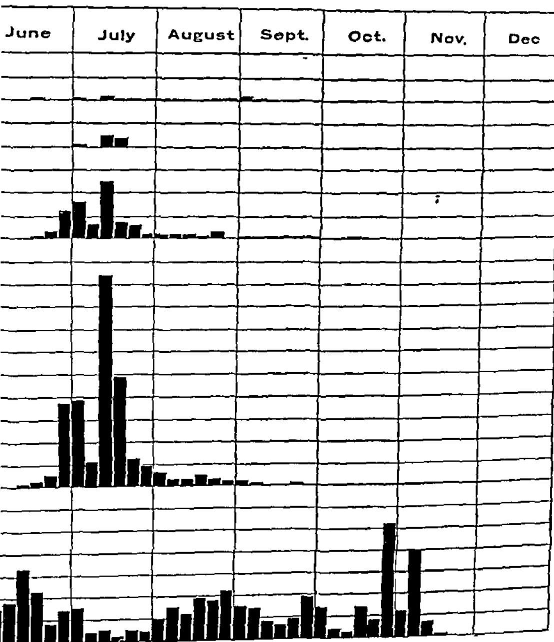


Chart V.

Yamato Cho, during 1925



Collection

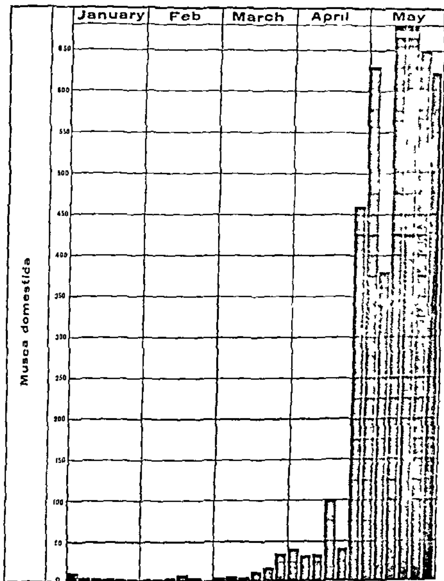
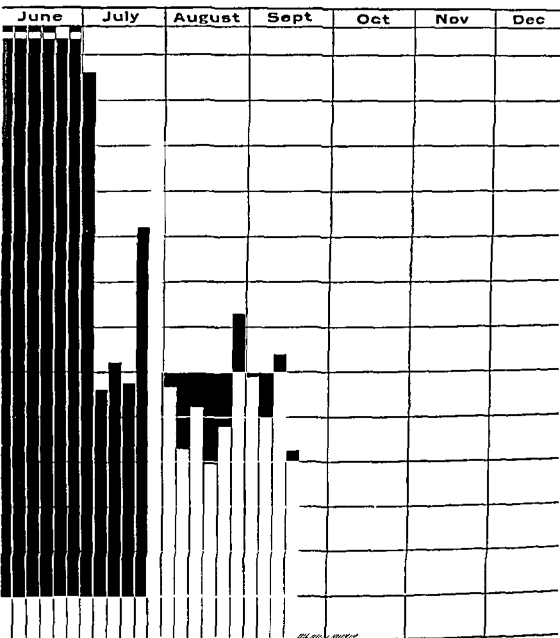

January
Feb
March
April
May
Musca domestica
550
500
450
400
350
300
250
200
150
100
0

CHART VII

in Fusan during 1927



according to the lowering of air temperature After November they disappear from most places being seen only in certain special houses

(B) *Other House-occurring Flies* Other species of flies were collected in relatively less numbers and it cannot be stated exactly as to their prevalent season I will only present briefly the following points —

Fannia (*F canicularis F scalaris* and others) and *Muscina stabulans* increase later than *Musca domestica* and they diminish sooner than the former

Sarcophaga (most flies being *S irrequiata*) and *Lucilia* (most being *L sericata*) prevail in the warmer season from June to August and they disappear usually at the middle of October

Overwintering of Flies In the former reports (that is 6th to 10th in the above list) I have discussed the overwintering of flies Adding some new data I will summarize the findings in the following conclusions —

Species which overwinter in the adult stage

1 *Musca domestica* It is not rare in winter in certain houses of Korean style and it propagates often during this season Further many female flies survive the whole winter season

2 *Muscina stabulans* Female flies hibernate only

3 *Calliphora lata* and *C erythrocephala* They propagate occasionally in winter and some female flies seem to survive the whole winter season

4 *Fannia canicularis* and *F scalaris* Some adult flies can survive whole winter season

Species which overwinter in pupal or larval stage

1 *Sarcophaga irrequiata* Hibernate in the pupal stage Some remain over eight months in this stage

2 *Stomoxys calcitrans* Hibernate in the pupal stage

3 *Lucilia sericata* This species does not hibernate but prolongation of the larval and the pupal stage occurs in the winter season The duration of this prolonged development often exceeds four months

4 *Fannia canicularis* Some hibernate in pupal stage

METEOROLOGICAL CONSIDERATIONS

Most of the meteorological data are taken from the reports of the Meteorological Observatory of the Government General of Chosen

1 Air Temperature The monthly air temperature in Keijo is shown in Chart VIII adding that of Tokio for comparison

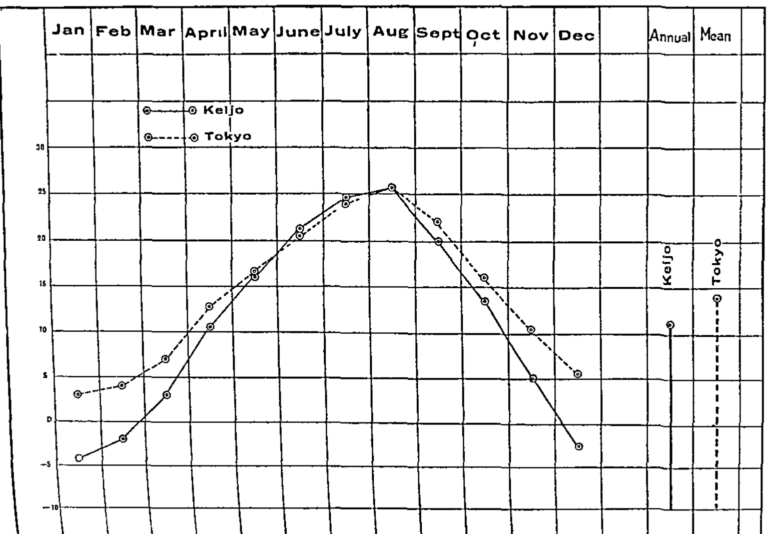
The chart shows that in winter the temperature in Keijo is much lower than in Tokio while in summer it is about same

2 Relative Humidity of Out-door Air The monthly average humidity is shown in Chart IX

Generally the air in Keijo is very dry but in July it has a higher humidity than that of Tokio

3 Precipitation The monthly average precipitation in Keijo is shown in Chart X

CHART VIII
Air Temperature.



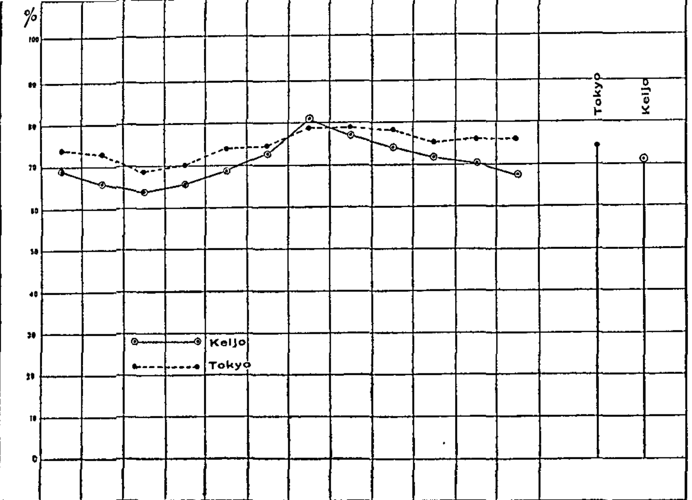
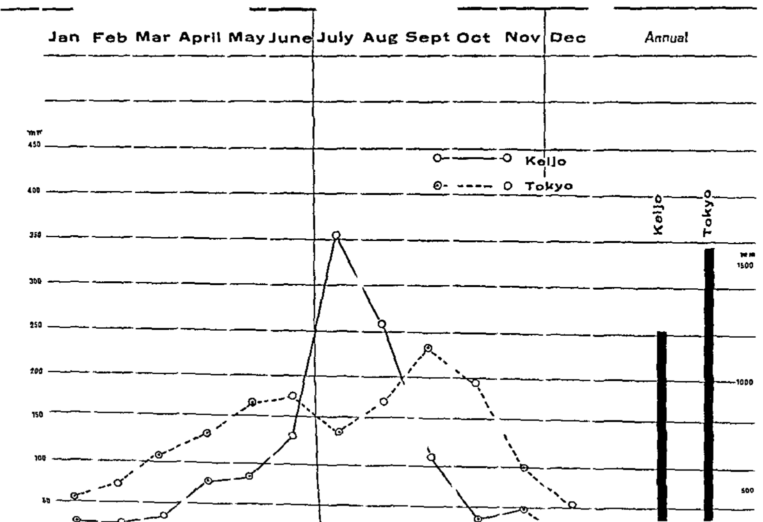

%
100
90
80
70
60
50
40
30
20
10
0
Keijo
Tokyo
Tokyo
Keijo

CHART X.

Amount of Precipitation



The total precipitation in Keijo is lower than that in Tokio while it is higher during July and August

We lack data as to the in door temperature and humidity for Chosen In Chosen a special method of heating the Ondoru is used in all houses of Korean style From the data we get in the laboratory i e Chart I it can be assumed that the Korean house in winter may be relatively high in temperature while very low in relative humidity

CONCLUSIONS

The results of my researches can be summarized in the following conclusions —

1 Experiments in the laboratory show that the flies survive longer and produce more eggs in dry and hot weather and adverse results are obtained in lower temperature and wet conditions

2 Counting of adult flies in nature shows that the flies are abundant two times in the year later spring and early autumn and they are not rare in winter also

3 Generally the climate in Keijo is dry except July and August

4 From these data it can be concluded that the seasonal prevalence of flies in Keijo and other parts of Chosen is affected by the climate of that locality in the dry and warmer season the flies being numerous while they are diminished in mid summer the season of wet weather * Hot and dry air in the rooms of Korean style during winter favours the overwintering of adult flies which results in early prevalence in spring

5 This method of investigating the seasonal prevalence of flies may contribute to research on the same subject in other localities

* In this article I have dealt only with the life of adult flies I will mention here briefly the account of fly larvæ In a mass of putrefying material, the breeding place of fly larvæ often it is not favourable for larvæ in hot season on account of the higher temperature and the want of oxygen in the interior of the mass In the cooler season the lowering of the temperature of the mass favours the life of fly larvæ These conditions seem to exaggerate remarkably the prevalence of flies in spring and autumn as compared to summer

DISCUSSION

Mr I Senior White (Bengal) Dr Kobayashi states that hot dry weather favours the oviposition and longevity of *M domestica* This is not so in the case of *M nebulo* of the Indian plains but on referring to the temperature charts, I notice that his maximum temperatures are many degrees below those we experience —which may account for the differences

I am very sorry that Dr Kobayashi has not specifically determined many of the other species he has charted In *sarcophaga* I have recently shown that there are species favoured both by dry and wet weather whereas I notice that Dr Kobayashi only records *sarcophaga* in wet weather

HELMINTHOLOGY

ENDO PARASITES DU TARABAGAN

PAR

LI YUAN PO

Service de prophylaxie de la peste de la Mandchourie

Introduction

THURSDAY DEC 8TH 2 TO 4 P M

On sait maintenant que le Tarabagan est la source de la peste pulmonaire dans les régions du Nord de Mandchourie et de la Sibérie

Chaque année le N M P P Service est approvisionne d un grand nombre de ces animaux capturés vivants en Mongolie pour les recherches afin d éclaircir la question d épidémiologie de cette maladie Comme nous faisons partie de ce service nous avons eu l occasion d examiner les parasites intestinaux des Tarabagans Cet article est le compte rendu succint des résultats que nous avons obtenus pendant ces dernières années

Description des Especes Trouvees

I —*Ascaris* sp

(Voir 'Report of the N M P P S' de 1927)

Cet Ascaris a été aussi vu par Monsieur Sukneff de Tcheta en 1923 Cette annee nous en avons rencontré deux chez un Tarabagan et dix chez un autre

II —*Entamoeba* sp

Cette amibe ressemble beaucoup morphologiquement à l Entamœba coli Son diametre est de 24 30 μ Celui du noyau qui prend fortement les colorants est de 3 à 5 μ environ On distingue très difficilement l endoplasme de l ectoplasme Ses mouvements sont très lents Nous avons souvent observé des Enteromonas ingérés par cette amibe quand ils coexistent dans l intestin d un meme Tarabagan

III —*Enteromonas* sp

Nous avons vu dans les selles des Tarabagans présentant un syndrome dysen térique un flagellé qui ressemble à l Enteromonas hominis da Fonseca Cet Enteromonas est de forme spherique ou ovalaire et animé de mouvements extremement rapides connus sous l expression rapid dancing movements de Wenyon et O Connor Il change de forme d une façon brusque et fréquente Il n a ni

cytostome ni membrane ondulante Les jeunes flagelles presentent a leur partie postérieure un prolongement a extremité pointue long de 6 a 8 μ La forme prékystique mesure 16 a 18 μ de longueur et renferme dans son protoplasme de nombreuses granulations tres chromophiles ce dernier caractere differencie des autres Enteromonas Le noyau est situé a la partie toute antérieure du corps La partie entre le karyosome et la membrane nucleaire ne prend aucun colorant Du blépharoplaste situé en avant du noyau partent quatre flagelles dont trois antérieurs sont libres et une posterieure passe le long du corps avec lequel il est intimement en contact Les flagelles se colorent difficilement Parfois on ne voit qu une ou deux flagelles anterieures au lieu de trois et la flagelle postérieure ne se colore que dans sa partie intra protoplasmique La forme prekystique renferme des masses de glycogene une grande ou bien trois ou quatre petites décelables par le Lugol double ou par la méthode de Best

(a) *Culture* —Ces Enteromonas contenus dans les selles de Tarabagans peuvent etre conserves pendant plus d un mois dans du sérum artificiel normal et a la température de 20°C Nous n avons pas pu observer les formes de division

(b) *Milieu de Musgrave Clegg* —Quand on les ensemence dans ce milieu à la température de 37°C dès le lendemain on peut rencontrer nombreuses formes de division de ces flagellés La dégénération sera retardee a la temperature de 25°C environ Dans cette condition ces flagelles commencent a se diviser au bout de 2 ou 3 jours

(c) *Milieu ovomucoide de Hogue*

(d) *Serum de Tarabagan* —Sérum 1
Solution salée a 1½ pour cent 10

Dans ces deux derniers milieux (*c* et *d*) ils se multiplient tres rapidement mais par contre la dégénérescence est aussi précoce on ne peut donc les conserver dans ces milieux aussi longtemps que dans le milieu de Musgrave Clegg

Role Pathogene

On trouve constamment ces flagelles en plus ou moins grand nombre dans les selles de tout Tarabagans atteint de syndrome dysentérique mais on ne peut dire à l heure actuelle si ces flagelles sont la cause meme de ce syndrome chez les Tarabagans ou bien si ce ne sont que des germes de sortie Cette question pour etre élucidée demande des recherches ultérieures

Conclusion

En resumé on peut rencontrer dans les selles de Tarabagans

1 Ascaris sp
2 Entamœba sp
3 Enteromonas sp

En outre on note parfois l existence de kystes ressemblant à ceux de Blastocystis hominis

DOCUMENTS STATISTIQUES SUR LES HELMINTHIASES A PONDICHÉRY

PAR

MAJOR V G F LABERNADIE,

Chef du laboratoire de Pondichery Establissements Français l'Inde

Voici quelques chiffres relatifs aux sept mois qui se sont écoules entre le 1 er Mars et le 30 Septembre 1927 Durant cette période, 1,075 recherches soit a l'etat frais soit apres coloration ont été exécutées sur 700 échantillons apportes au Laboratoire Si nous extrayons de ce dernier chiffre les examens de controle thérapeutique, il reste 565 selles examinées pour la premiere fois

Parmi ces 565 primo examens 291 étaient positifs ce qui montre que 51 pour cent de la population prise en général est parasitée par les helminthes

La moitié environ de ces primo examens était uni parasitée, l'autre moitie était bi ou tri parasitée

Voici le detail du *pluriparasitisme* { selles a 3 parasites 17 soit=435
,, 2 ,, 110 parasites
,, 1 ,, 164 *au total*

En tenant compte de ce dernier chiffre vis a vis du nombre des primo examens on obtient *l'index parasitaire* $\frac{435}{565}$= 77 pour cent qui mesure en somme l'importance des reservoirs de virus

La frequence des diverses especes rencontrees est a étudier (1°) par rapport au nombre des primo examinés (565) (2°) par rapport au nombre des positifs (291)

Espèces	Sur 565	Sur 291
	Pour cent	Pour cent
Ascaris 158 fois	28	54
Ankylostome 122 fois	21	42
Trichocéphale 147 fois	26	50
Anguillule 8 fois	1 5	2 5

Les *associations parasitaires* les plus fréquentes sont —

Ascaris et Trichocéphale .	54 fois.
Ankylostome et Trichocéphale .	27 fois
Ascaris et Ankylostome	21 fois.
Ankylostome, Ascaris et Trichocéphale ..	14 fois

STRONGYLOIDOSIS AND ITS TREATMENT WITH GENTIAN VIOLET

BY

KWA TJAON SIOE

Weltevreden

In the "Handbuch der Tropenkrankheiten" (2nd edition) Looss says "Al the pathological importance of the *Strongylus stercoralis* a divergence of opin still exist This divergency is chiefly caused by the fact that uncomplicated c of infection relatively seldom occur Mostly one finds besides the Strongylus other intestinal parasites which are more considered as a morbific cause whe the Strongylus more as a Nebenbefund"

Strongylus (Anguilula) stercoralis belongs to the nematodes namely to family of the *Angiostomidæ* Particulars about this worm one finds in all manu so I need not refer here to them

The existence of this kind of worm among the Chinese population of Bata is almost negligible In the period of five years extending to more than 2 fæces examinations mostly from the Chinese population of Batavia I found o five times the larvæ in the fæces Out of these five one has come from a nat lady and another from a Chinese girl from Pandeglang So I can safely supp that no more than 1 or 1 5 per mille of the Chinese population of Batavia infected with it

Out of the five cases I can consider two as not pathogenic The first of th two came to me with sanguinary diarrhœa and was first treated for his amœ dysentery and afterwards for Ascaris When the amœba and Ascaris eg disappeared from the fæces the patient did not complain any more and yet t fæces was still thickly populated with Anguilula larvæ My second case in wh I can consider strongyloid larvæ as non pathogenic occurred in a girl from Pand lang She came to me with abdominal complaints pains in epigastric rumbli in the stomach diarrhœa after every meal with undigested food in the fæces the stool I found eggs of Ascaris Anchylostoma and Strongylus larvæ

Oleum chenopodii expelled the Ascaris worms and partly the hookworn After one weel a fæces examination showed only sporadic hookworm eggs where the number of Strongylus larvæ seemed to be uninfluenced A second oleu chenopodii administration resulted in there being after a week only Strongyl larvæ in abundance After the expulsion of the hookworms this girl had complaints any more

The second cure for the hookworms was followed reluctantly because both patient and her parents could not understand why one had still to take that untasteful oil if she felt quite better

A year later I got this patient again at my consulting hour with complaints in the stomach A fæces examination gave as result Ascaris eggs and larvæ of Strongylus A santonine cure freed her from complaints she was free from Ascaris eggs but I found the larvæ in the dejecta

Out of the five cases, there are three which I consider as strongyloidosis of pathogenic importance

The first of these was a native lady married to a European This lady came with stomach aches which now and then had the character of gripes The fiercest aches she felt in the right part of the abdomen above the umbilicus

A short description may follow here —

A fat anæmic lady about 30 years of age nervous when speaking Since a few years under treatment for the stomach aches now and then interchanged with vomiting and anorexia by several physicians at Sourabaya and Makassar She has had three children of which one alive The other children had died of fever and coughing none of the three children had had convulsions The lady informed further she had never had a miscarriage Physical examination Lungs nothing particular The heart action was accelerated with anæmic murmurs The spleen was not to be felt and the liver was palpable by deep inspiration with a sharp and smooth edge not painful The belly has no défense musculaire She had no jugular glands nor bicipital glands

Reflexes of the pupils were normal and patellar reflex only very weak and difficult to be excited

No paræsthesia

Blood no malaria parasites eosinophilia Tallquist 50 per cent

Urine nothing particular

Fæces hard consistence only Strongylus larvæ no other known pathogenic contents of the intestines

No blood was taken from this patient for the Wassermann test but a luetical infection was denied by her husband

This lady I have treated with santonine extr felicis maris glycerine oleum chenopodii in turns with different intervals but without any result The existence of the liver enlargement suggested me to give her five successive emetine injections but all in vain

With all these medicines besides roborantia I could see not a single result the Strongylus larvæ were still to be found as easily as before and the patient still kept her complaints The complaints became worse some months later when the menstruation stayed away

As she had been treated by me without success for more than three months and was only relieved from her complaints by taking a powder with belladonna *and papaverine I persuaded her to consult another physician The latter treated* her with emetine injections but also in vain

After some months she consulted me again She was pregnant these four months and complained of pains in the abdomen and in the back together with a bleeding out of the genitalia A gynæcologist drew some hours after the first signs of miscarriage a fœtus of about four months Though the lady was fed with

food rich in B vitamin and the bleeding during the abortion could not be called excessive she succumbed in the same night

The vomiting during the first month of pregnancy did not tend to improve matters as far as the treatment with B vitamin was concerned The death of this lady we could not explain as being caused by hæmorrhage The cause of death must also be sought in the beri beri heart The part of strongyloidosis in this case we cannot ascertain Analogically to anchylostomiasis, which forms a weakening in the power of endurance of the organism against injurious influences from outside, we can take it for granted that the presence of strongyloid worms in the duodenum is not conducive to the health of the patient The second case I shall mention here is definite proof of the pathogenic nature of the Strongylus worms

A Chinese boy 5 years old born at Batavia had suffered from May 1926 of diarrhœa now and then he had pains over the whole belly alternatingly His appetite was declining steadily and the boy was growing lean during the treatment A physical examination showed me nothing particular in the heart lungs liver spleen etc When examining the belly, the boy showed me the place of the pains he felt but this place varied every time There was no distinct défense musculaire Vomiting the boy did not do Defæcation four to six times a day thin as porridge grey yellowish of colour smelling very bad

Microscopically I found besides leucocytes a great many of vivid little worms of about 1 millimetre in length

Amœba cysts and worm eggs I could never find after repeated fæces examination

Thomas of the Leipzig clinic of Struempell says in his description and consideration of a case of strongyloidosis observed by him that from pathological anatomical researches it is evident that it will be a difficult task to exterminate the *Anguilula stercoralis* The various publications about the variety of means which are recommended show us again this difficulty

With this boy I have tried from June up to October several anthelmintica without any result I regularly examined the fæces and I always found the larvæ sometime more numerous sometime less numerous, but I have not yet stated one single time they were entirely expelled

Under the coverglass I have brought several medicaments as emetine tartras emeticus and colouring matters as blue of methylene eosine, gentian violet and further oleum chenopodii santonine, etc , in contact with the larvæ

A certain medicament killing the larvæ more quickly I could not state Under the coverglass I can see the larvæ die after two to four hours as well with aqua destillata as with one of the medicaments mentioned above beginning with diminishing mobility, than standstill , gradually the larvæ become hazy and dissolve in the surrounding mass not long afterwards I have kept fæces that I received in the morning till evening in order to examine the larvæ which I supposed would have become riper and would have changed from the rhabditic form to the filarial form but found the fæces free of larvæ and worms

The same Thomas and V Kurlov have also stated in their cases that the lack of resistance capacity and the great inclination to break would then be characteristic

for the rhabdonemata Thomas had success with the treatment of his case with big doses of thymol, viz 5 grammes on the first day and 10 grammes the second day So strong a treatment I dare not apply with my little patient because another older patient could not stand a dose of 3 grammes a day for three days running

With another new drug against anchylostomiasis of which Fuelleborn is an enthusiastic defender, the carbon tetrachloride I dared not make experiments on account of the toxicity of the drug and because Fuelleborn himself wrote that this drug had proved to be entirely powerless to expel the Strongyloides

Reed also wrote that carbon tetrachloride appears to have no effect against Trichocephalus and Strongyloides As chance would have it a colleague of mine called my attention to a publication by Faust about the treatment of clonorchiosis with gentian violet Gentian violet I used after reading Faust s publication for the treatment of clonorchiosis with alternating success

Without conviction of success as my little patient again and again came back at my consulting hour I wanted to take an experiment with the administration of gentian violet

The intravenous administration of the 1 per cent solution which I usually gave to my clonorchiosis patients I would not give to a small boy because a little technical mistake in injecting can cause painful infiltrates of the vein and these infiltrates can reach a considerable length So I decided to give the boy *per os* three times 1 tablet of 100 mg gentian violet a day These tablets are from Burroughs and Wellcome and are obtainable in tubes of ten tabloids

Should this medicament not help it would in my opinion also not harm for intravenous administration of 600 mg per diem during some days running was endured without any inconvenience by clonorchiosis patients

After three days the fæces was brought again to me the diarrhœa had ceased Motions occurred only twice a day fæces was soft and thick Microscopically I could find larvæ no more As I was afraid of a temporary disappearance of the larvæ I gave the patient the tablets again for three days Regular control two times a week in the beginning later once a fortnight, during more than six months always gave a negative result

The diarrhœa had disappeared appetite health etc returned and the boy grew again in weight

A third case was a lady of 28 years In November 1926 I got her under treat ment for a common five day fever After the rash had broken out the fever had entirely disappeared The lady still complained of violent pains in the epigastrium after the fever these pains she would also have felt often before her fever When examined she showed the pains between the umbilicus and the gall bladder region Défense musculaire was absent She had no icterus vomited now and then the vomit had as a rule a bitter taste The appetite alternated Motions were mostly slow and difficult, once in two or three days interchanged by diarrhœa several times a day

I treated her symptomatically thinking to have to do with neuralgic pains after a five day fever Fæces examination was attended with difficulties and only after some weeks could I persuade the patient and her family to send fæces to me In the hard fæces I found beside leucocytes only sporadic larvæ of Strongyloides After I had tried in vain with thymol salol, oleum chenopodii and glycerine I gave the patient the advice to take belladonna in combination with papaverine if the pains should be very violent

The pains did surely not come regularly every day, but they might be so violent that she shouted the whole house down and I thought of hysterical attacks for objectively I could find nothing on palpation Her reflex of the throat was positive After the successful treatment of my previous patient I gave her three times two tablets of 100 mg gentian violet a day

Nausea was the only disagreeable thing of this medicament At the disappearance of the Anguilula larvæ out of the fæces the pain did not come back any more

Thomas whom I have mentioned above has been able to state with the duodenal probe and with the X ray that in spite of a hypochlorhydria the sphincter pylori was in a spasmodic state and was impassable for the duodenal probe The X rays showed a distinctly hypertonic stomach after a meal which however did not show a slackened digestion Should the continual severe pains of the first and third patient be attributed to cramp of the pylorus ?

Summary

(1) There is a divergence of opinions about the pathogenicity of strongyloidosis In some cases this pathogenicity cannot be disputed
(2) With the common anthelmintica the Strongyloides cannot be killed without difficulties
(3) Gentian violet tablets administered *per os* can kill the worms within a short time
(4) The action of gentian violet is probably direct on the worms and *in vitro* it has not more effect than the other drugs against the larvæ

REFERENCES

MENSE — Handbuch der Tropenkrankheiten
ERICH THOMAS (1923) — Ueber Darmerkrankungen durch Strongyloides intestinalis *Deutsches Arch f Kl Med* Bd 141
FAUST and YAO KE FANG (1926) — *Arch f Schiffs und Tropenhygiene* Bd 30 Heft 9
OLIVIER and KANDOU — *Geneesk Tydschrift v Ned Ind* Vol LXVII No 1
KWA TJAON SIOE — *Ibid* No 2
REID (1924) — *Brit Med Jour* June

THE PRESENT STATUS OF *SAURICOLA* AND *ECHINOPHARYNX*

BY

GOBIND SINGH THAPAR, M Sc Ph D (Lond)

Offg Professor of Zoology The University Lucknow

CHAPIN (1924) described a new genus *Sauricola* obtained from the intestine of the Brazilian land tortoise *Testudo denticulata* Later the writer obtained another genus from the same host and named it *Echinopharynx* The validity of the latter has been doubted by Yorke and Maplestone (1926) who fused the two genera into one and since *Sauricola* was published earlier it has the priority These authors have not given any reasons for doing so and have taken the mathematical mean of the two original communications They have accepted the figures of Chapin and the account of Thapar (1925) and have thus further added confusion for the workers on nematology by mixing the accounts of both the genera into one It is therefore desirable to clear up the misunderstanding thus created

The writer obtained some material through the courtesy of the United States National Museum and thus had the opportunity of examining some of Chapin s material He finds that Yorke and Maplestone were not justified in fusing the two genera into one The following account of *Sauricola* is added in order to clear up the matter

I wish to state here that I feel deeply indebted to the authorities of the United States National Museum for the readiness with which they acceded to my request to send me the material for my examination

Description of Sauricola sauricola *Chapin* 1924

The body is elongated cylindrical The female is larger than the male

The cuticle is thick and distinctly annulated throughout Anteriorly it forms the mouth collar and bears circum oral papillæ The circum oral papillæ are usual in number and disposition

The excretory pore is situated ventrally behind the œsophagus and is situated in the centre of a depression of the cuticle It is 0 6 mm from the anterior end of the body The cervical papillæ are absent but their position is indicated by small conical protuberances of the lateral body wall on the sides of the excretory pore These do not extend beyond the surface cuticle and thus exhibit degenerate characters

The nerve ring surrounds the œsophagus in its posterior part and lies at 0 18 mm from the anterior end

The mouth leads into a shallow buccal cavity 0 05 mm long and is supported by a thick cuticular ring the buccal capsule It bears two rows of leaf like processes forming the leaf crowns, the corona radiata externa and the corona radiata interna each consisting of eighteen elements

The œsophagus is a short, cylindrical structure 0 3 mm long and has the diameter of 0 17 mm The œsophageal funnel is present and the cuticular lining is thick and bears a large number of conical spines projecting into its cavity There are three œsophageo intestinal valves projecting into the intestine from the posterior end of the œsophagus The intestine is cylindrical and is lined internally by very thick cuticle

Female Characters The body of the female is 10 mm long and bears a small conical tail The body narrows slightly towards the posterior end The anus is situated at the posterior extremity of the body, a little in front of the base of the tail and leads up into the rectum lined with thick cuticle

The vulva is situated in front of the anus, the distance between the two being 0 1 mm It enters into a short vagina 0 2 mm long Anteriorly it divides into two horns that are long and run parallel to each other and narrow up in front to lead into long uteri The uteri are full of eggs at varying stages of development They extend as far forward as the middle of the body and lead into their respective ovaries that are narrow coiled tubes

The eggs are oval and show varying degrees of segmentation They measure 0 13 mm by 0 06 mm

Male Characters The male is only 8 mm long Its bursa is composed of three lobes that are not distinctly cut up, but they can be distinguished by the presence of slight notches between the dorsal and the lateral lobes Ventrally the cuticle of the body wall is also expanded to form a pre anal bulla The various lobes of the bursa are supported by the bursal rays The rays of the lateral lobe arise by two stems, and in addition there is a pre-ventral ray that supports the pre anal bulla and is therefore the pre bursal ray The ventral rays arise by a common stem but immediately divide into two parallel rays the ventro ventral and latero ventral The lateral rays consist of three complete rays arising by a common stem This soon divides into two unequal lobes the ventral bearing the externo lateral ray and the dorsal bearing two parallel rays the medio lateral and postero lateral This lobe also gives a stout but rudimentary extra lateral ray resembling that of *Echinopharynx* and the *Œsophagostomum radiatum* The dorsal ray arises independently and soon after its origin gives off a narrow externo dorsal ray reaching the edge of bursa and itself runs backwards in the dorsal lobe of the bursa It divides twice to give rise to two dorsal rays on either side of the median dorsal line

The genital cone is fairly prominent but does not bear any flagelliform processes found in the case of the genus *Echinopharynx*

There are two spicules of equal length, pointed at the free end They are 2 38 mm long and are alate An accessory piece 0 07 mm long is present along the dorsal wall of the cloaca

It would thus appear that the above account of *Sauricola* agrees with the account given by Chapin for his genus the only difference being in the nature of the spicules that might have been missed by that writer due to oversight The generic diagnosis of *Sauricola* should therefore be read thus —

'Shallow buccal capsule surrounded by two leaf crowns mouth collar limited behind by an annular constriction Excretory pore post œsophageal cervical papillæ and cervical groove absent Œsophagus cylindrical armed with spines Bursa short not separated into dorsal and lateral portions Pre anal bulla present enclosing pre bursal papillæ Ventral rays distinct to the base a small dorsal process arises from the main trunk of the lateral rays Dorsal ray quadridigitate spicules long slender and *alate* In female vagina is short ovejector and sphincter muscles not developed uteri two and parallel'

Let us now find out the points of difference of this genus from the diagnostic characters of the genus *Echinopharynx* I have already (1925) given an account of the latter and its distinguishing characters are —

'Shallow segmented buccal capsule *having a single leaf crown* forming a corona radiata, short œsophagus provided with spines Excretory pore post œsophageal *narrow cervical papillæ present* also post œsophageal intestine with three *intestinal diverticula* Pre bursal papillæ long and ray like small extra lateral ray present alate spicules In female vulva surrounded by a *shallow marsupium*, short vagina with two horns uteri parallel and ovejector absent

Looking at the above diagnostic characters of the two genera it would appear that they differ from each other in the following chief characteristics

Sauricola	*Echinopharynx*
Double leaf crown	Single leaf crown
Buccal capsule shallow	Buccal capsule segmented
Cervical papillæ absent	Cervical papillæ present
Intestinal diverticula absent	Intestinal diverticula present
Vulva opens directly to the surface	Vulva surrounded by a shallow marsupium like structure

The differences indicated are sufficiently prominent to deserve special consideration The presence of the intestinal diverticula cervical papillæ and the female marsupium in *Echinopharynx* are sufficient to justify the creation of a new genus by the writer These characters are not exhibited by any other genus of the Strongylidæ The character of the presence of the diverticula in connection with the alimentary canal has already been recognized in another family of the nematodes the *Heterocheilidæ* I find no reason why it should not be extended here also

The synonymy of the two genera was brought to my notice by Professor Yorke in a private communication soon after the publication of my paper on *Echinopharynx* when he very kindly drew my attention to Chapin's paper. On looking through this communication the writer was convinced that he was rightly describing the form under a new genus. This was communicated to Professor Yorke with the writer's opinion on the matter. Notwithstanding this Yorke and Maplestone (1926) mention that the two forms are identical and placed *Echinopharynx* synonym to *Sauricola*. They have given no reasons for doing so. The writer at first had some hesitation in contradicting their statement as he was under the impression that the opinion expressed by them was the result of their 'personal observation' on the material but on a careful study of the account given by them it appears that it is not so. While calling the two genera synonym to each other they throw out the description of Chapin and accept his figures and thus add confusion in the matter. The following points would clearly explain it. The figures indicate a complete absence of the intestinal diverticula but in the description mention is made of the presence of 'three pocket shaped diverticula directed posteriorly.' The figures are clear in showing double-leaf crowns while the description indicates only a single corona radiata of 36 elements. It would thus appear that there is incongruity between the figures and the text and it is not known how the authors would explain it except by stating that they themselves are in doubt as to the exact position in the matter of the fusion of the two genera into one. One further point may be mentioned here. They have given in the list of other species the name *Sauricola echinopharynx* which is the same as the *Echinopharynx echinopharynx* Thapar, mentioning it as a second species but in the foot note they say 'possibly this species (*Sauricola echinopharynx*) is identical with *S. sauricola* but the spicules are stated by Thapar to be 4.05 mm in length as compared with 2.36 mm in *S. sauricola*.' It appears that they even doubt the validity of recognizing it as a second species. The only difference they could detect between the two forms was as regards the size of the spicules and nothing beyond it. The writer has given a complete account of *Echinopharynx* and has just mentioned the salient features in which it differs from the genus *Sauricola*. He now hopes that the authors will be able to recognize the differences that are based on the observations of the material obtained from both sources. The writer is now strongly convinced that the two forms described in two distinct communications are not only different but actually belong to two different genera. Both Chapin and the writer were correctly describing their forms under different genera and Yorke and Maplestone had no justification in fusing them together.

Although the two genera are fundamentally different they do show certain resemblances to each other, thus for instance they are both obtained from the same host, they both possess spines in the œsophagus, they both have pre bursal papillæ and an extra lateral ray of variable size, and they both show the presence of alate spicules and absence of an ovejector. This indicates that there can be several genera that may exhibit similarities with one another and it would

indicate that a probable phylogenetic relationship may exist between them and may justify their inclusion under a common sub-family Two common features which they share with another genus *Kiluluma* are (1) absence of an ovejector, and (2) presence of an extra lateral ray Therefore they may be included under the sub family *Kiluluminæ* created by the writer originally for *Kiluluma* This sub family diagnosis is it is admitted based on sexual characters and is therefore not a natural grouping but such characters have been accepted in other groups like *Oxyuroidea* as convenient for diagnosis and may be retained here in this case as well at any rate till a more satisfactory solution of placing the classification of nematodes as a whole on the natural system of classification has been found The genera may be recognized thus —

(1)	Œsophagus armed with spines	2
	Œsophagus unarmed	*Kiluluma*
(2)	Intestine with intestinal diverticula	*Echinopharynx*
	Intestine without such diverticula	*Sauricola*

REFERENCES

CHAPIN E A (19-4) Nematode Parasites of the Brazilian Land Tortoise *Testudo denticulata* *Proc U S Nat Mus* Washington Vol LXX p 1

THAPAR G S (1924) On Kiluluma Skrbn a genus of Strongylid Nematodes Parasitic in the African Rhinoceros *Jour Helm* Vol II pp 209 238

Idem (19) On the Morphology and Systematic Position of *Echinopharynx* a new genus of bursate nematodes from *Testudo tabulata* *Ibid* Vol III pp 19 3

YORKE, W and MAPLESTONE P A (1926) The Nematode Parasites of Vertebrates, J and A Churchill London p 61 6

Explanation of Plate X.

Fig. 1. Anterior end of the male

" 2. Posterior end, ventral view of the female.

" 3 Posterior end, side view of the female.

" 4. End-on view of the head, showing buccal capsule and leaf-crown.

" 5 Male bursa, lateral view.

" 6 Male bursa, ventral view

PLATE X

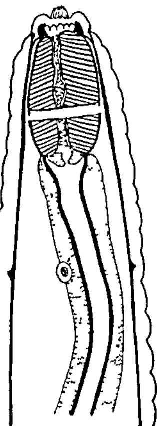


Fig 1

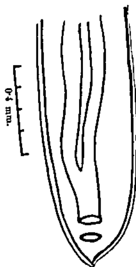


Fig 2

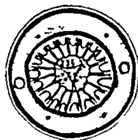


Fig 4

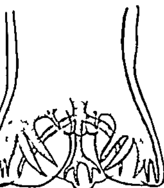


Fig 6.

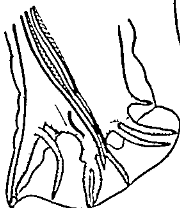


Fig 5.

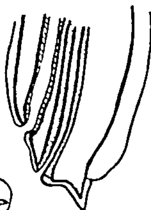
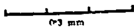


Fig. 3.



STUDIES ON PROPHYLAXIS OF CLON

BY

KANJI NAGANO M D
The Kitasato Institute for Infectious Diseases Tokio
Okayama

PROPHYLAXIS of clonorchiasis has up to the preser
authors but all of them suggested only passive measure
As to the active measures to destroy the Clonorchis so
practicable one at present

The life circle of *Clonorchis sinensis* has been made
and Muto (1918) Therefore it is needless to discuss th
exterminated if the life circle could be cut off anywhere

The measure however must be effective harmle
applicable in practice

For obtaining such a measure I have made biolo
intermediate host *Bythinia striatula* var *japonica* as I
of Bythiniæ might be the best way to attain the end

Salient points of bionomics of Bythinia which
follows —

(1) The districts which Bythinia inhabits are
favours the stagnation of water such as slow flowin
shores and so on In canals which dry up during wint
rarely found

(2) During winter it hibernates in the mud It
about the end of March and to hibernate from about th

(3) It is oviparous and it seems to be diœcious

(4) Its breeding season is from the end of April til
breeding climax is June and July It spawns on an aver
at an interval of about eight days Accordingly it may
lays about 500 to 600 eggs a year

(5) It lays spawn mostly on water plants and al

STUDIES ON PROPHYLAXIS OF CLONORCHIASIS

BY

KANJI NAGANO M D

The Kitasato Institute for Infectious Diseases Tokio and the Medical College Okayama

PROPHYLAXIS of clonorchiasis has up to the present been studied by senior authors but all of them suggested only passive measures to prevent the infection As to the active measures to destroy the Clonorchis so far as I know there is no practicable one at present

The life circle of *Clonorchis sinensis* has been made clear by Kobayashi (1910) and Muto (1918) Therefore it is needless to discuss that the Clonorchis would be exterminated if the life circle could be cut off anywhere even at one place

The measure however must be effective harmless economical and easily applicable in practice

For obtaining such a measure I have made biological studies on the first intermediate host *Bythinia striatula* var *japonica* as I thought the extermination of Bythiniæ might be the best way to attain the end

Salient points of bionomics of Bythinia which I have made out are as follows —

(1) The districts which Bythinia inhabits are generally low land which favours the stagnation of water such as slow flowing canals swamps and lake shores and so on In canals which dry up during winter or have no outlet it is rarely found

(2) During winter it hibernates in the mud It begins to be active from about the end of March and to hibernate from about the end of October

(3) It is oviparous and it seems to be diœcious

(4) Its breeding season is from the end of April till the end of August The breeding climax is June and July It spawns on an average of 36 eggs at one time at an interval of about eight days Accordingly it may be said that one Bythinia lays about 500 to 600 eggs a year

(5) It lays spawn mostly on water plants and also on bamboo brushwood and on smooth pieces of crockery which are placed in the water provided they are not covered thickly with incrustation

(6) Its spawn hatches out in about 14 days in May and in about 11 days after July

(7) Young Bythiniæ are good food for fresh water fishes as the shell of the young ones especially within about ten days after hatching is very soft

(8) Abundance of water plants is a good defence for young Bythiniæ from natural enemies, such as fresh water fishes

(9) In my examination in Okayama district there have been found up to the present ten kinds of cercariæ in Bythinia Nine of them or all except the Clonorchis are almost or quite indifferent to human beings

(10) Bythiniæ which harbour the cercariæ, deposit no spawn except in very rare instances the cause of which fact seems to be a mechanical disturbance on the gonad Furthermore such infected Bythinia die generally earlier than non infected ones

On the ground of the above facts I recommend the following two measures to destroy the Bythiniæ

(1) The measure to destroy the Bythiniæ by removing of the water plants and simultaneously putting in bamboo or brushwoods taking into consideration the breeding season and the number of days required for hatching

During the breeding season in 1926 I made the experiment in the field and proved it to be a wonderfully effective method

This is a reason why by this measure numberless spawn laid on water plants bamboo or on brushwoods are taken away from the breeding places and young Bythiniæ hatching out of the spawn which remained are eaten by fishes because once most of the water plants are removed the fishes are able to swim very freely to find them

The removal of the water plants *in the canal is done in summer three to four* times yearly as a routine work in Okayama district but it is done at present only for the purpose to make canal water flow easier If the advantage is taken of this opportunity so as to serve the new purpose a great deal of additional labour can be saved

(2) The measure to destroy the Bythiniæ by means of parasitic castration As above mentioned Bythiniæ infected by cercariæ deposit no spawn in nearly all cases Therefore we can recognize *theoretically* that the Bythiniæ might be exterminated if we increase the number of some harmless trematodes which are harboured in Bythiniæ and if we exert our efforts to make the miracidia to infect *Bythiniæ up to 100 per cent The experiment in the field regarding* this measure *is now going on* so details will be reported later

In short both the above measures are satisfactorily endowed with the conditions we require as the practical measure If these two measures are executed for years continuously I presume that the Bythiniæ might be exterminated because by these measures the balance in the natural world by which the existence of all living things may continue is severely and constantly disturbed and even though *they might not be exterminated completely,* it has been experimentally proved that they are strikingly decreased even by the first measure alone

In closing, it may be mentioned that the second measure can be applied to destroy any other snails which act as intermediate hosts of human trematodes

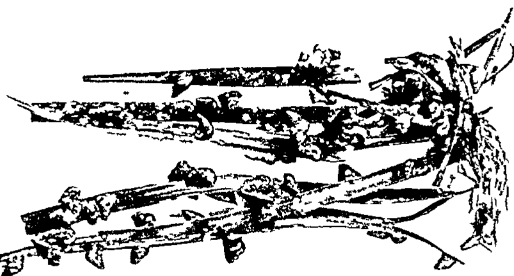


Fig 1

Fig 2

Explanation of Plate XI

Fig 1 Abundance of Bythiniæ in favourable breeding area in Okayama prefecture

„ 2 Spawn of Bythinia laid on water plants bamboo etc in natural state

EXPLANATION OF PLATE XII.

Fig 3. Spawn of Bythiniæ laid on bamboo after the mowing (*in experiment*)
" 4 Spawn of Bythiniæ laid in bamboo basket (*in experiment*)

PLATE XII.

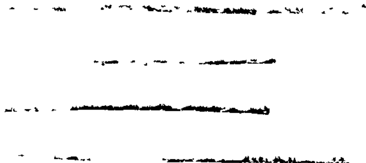


Fig 3.

Plate XIII





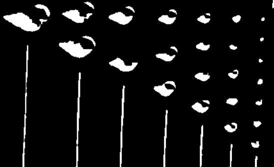




Fig. 5.

THE CORRELATION BETWEEN THE SIZE OF HOOKWORM EGG COUNTS AND THE DEGREE OF ANÆMIA IN TWO GROUPS IN SOUTHERN INDIA *

BY

J F KENDRICK, M D, D P H

Madras

THE effect of the hookworm on its host is a subject about which there seems to be great difference of opinion In the belief that light infestations may be responsible for injury that is masked in the average individual by his ability to compensate for it it is argued on the one hand that infestation with the fewest possible number of worms is detrimental and that the parasites should be dislodged whenever and wherever detected On the other hand there are those who contend with just as much sincerity and probably more reason that even where a high *percentage of the population is infested treatment should be resorted to only when* it is possible to demonstrate that the parasites are producing physical deterioration

Theoretically it is conceivable that moderate numbers of these parasites produce a constant drain upon the energy and vitality of the host that becomes manifest only under conditions of stress and strain It is conceivable also that the effort on the part of the human system to compensate for the injury resulting from a light infestation might be a dominant factor in determining the course of a grave illness Those who have made careful observations in the tropics however *are aware that there are extensive areas where more people are infested than not* yet there is no possible way of recognizing the fact except by the finding of a few ova in the stools Such persons appear to be perfectly well and happy and compare favourably with their non infested associates

Without further reference to the merits of either of these contentions it is proposed to show the results of observations on two groups of Indians made during parts of the years 1926 and 1927 Accepting anæmia as the most constant and undoubtedly the most prominent sign or symptom associated with hookworm *infestation, an attempt has been made to show what correlation there is if any* between the hæmoglobin content of the blood and the degree of infestation as revealed by egg counts

---

* ...hich this paper is based were conducted with the support ... Health Division of the Rockefeller Foundation and the

The writer desires to record his grateful thanks to Mr E R Sundararajan M A, for many suggestions and help in the preparation of the statistical part of this report

Methods of Procedure

The egg counts were made by a slight modification of Stoll s method The modification consisted of making counts without a coverslip over the preparation In all other respects the technique was exactly the same as that recommended by Stoll (1923) except that 5 grammes raised to 75 c cs with decinormal sodium hydroxide (Stoll and Tseng 1925) was used instead of three grammes raised to 45 c cs The weighing and diluting was always done by a government qualified compounder It is believed that with the arrangement devised to prevent currents of air from striking the preparation on the slide counts can be made more accurately without a coverslip than with one We usually found that the preparation would remain undisturbed if the windows were closed at the bottom and opened at the top Two counts were made from each tube on about half the cases in the first group The average of these two counts was taken as the count for that particular individual On the remainder of the cases only one count was made On the other group (275 persons) from two to twenty counts were made The average of all these counts was taken as the accepted egg count All the stools were classified as formed or unformed no diarrhœic stools being accepted It is believed that the term unformed used here refers to stools that would be classified as mushy by Stoll All the counts presented in this paper are on the formed basis' (Stoll 1924) except where it is otherwise expressly stated

The first group referred to above consisted of 13 303 persons—men women and children These were examined at the Ceylon Emigration Depot Mandapam Camp Thousands of Indian coolies pass through this camp annually *en route* to Ceylon where they are engaged on the tea and rubber estates This element of the population is known to carry the heaviest hookworm burden of the respective districts from which they come and if hookworm disease is causing serious harm in southern India it ought to be possible to demonstrate it at this camp These labourers are extremely poor In their villages they are badly housed underfed and their surroundings are highly insanitary In spite of this the health of the average cooly appears to be comparatively good In our investigation every reasonable precaution was taken to eliminate those who were suffering or had recently suffered of any disease other than hookworm that might be responsible for a reduction of the hæmoglobin content Insufficient and poorly balanced diet probably was responsible for some degree of impoverishment of the blood in this entire group

The second group consisted of 275 men examined at the Madras Penitentiary Egg counts were made on each of these for several consecutive days following their admission These completed each man was treated the stools collected and washed

through a fine sieve and the worms were collected and classified Treatment was continued until no worms were present in the resulting stools and no ova could be detected in the fæces by Lane's method a fortnight after the last treatment These worm counts were made under almost ideal conditions As a rule the men were locked in separate rooms on the day of treatment and all the resulting stools were collected during the following sixty hours A dose of magnesium sulphate was given in the early morning of the two days following treatment to insure the removal of all worms On days when the number to be treated exceeded ten it was necessary to place several men together in a ward where they were carefully watched as a safeguard against loss or mixing of stools As these prisoners had n opportunity of losing or accumulating any worms in the interval between admission and treatment the egg counts and worm counts should be just the same as if the work had been done in the villages from which they came The group contained representatives of all the social divisions both Hindus and Mohammedans By occupation most of them were cultivators or other out of door labourers Persons suffering from any detectable disease other than hookworm were rejected

Hæmoglobin readings were taken from every person in the two groups These were read to the nearest five per cent on the Tallquist scale and the readings on the two groups were made by different men

Statistical Analysis of the Data

As previously described the groups consisted of 275 persons (Group B) studied at the Madras Penitentiary and 13 303 persons (Group A) studied at Mandapam Camp Whereas it was only possible to egg count the large number in Group A *egg counts and complete worm counts were made on Group B* The species of worms recovered from this group are shown in detail in the following table —

Ankylostomes		Necators		Total		Grand Total
Male	Female	Male	Female	Male	Female	
211	251	3 541	4 302	3 752	4 553	8 305

The percentage of Ankylostomes to total worms is —

(*a*) 5 5 per cent (ratio female to female)

(*b*) 5 6 per cent (ratio male to male) and

(*c*) 5 6 per cent (ratio Ankylostome total to total worms)

As a rough index of the relative number of Necators to Ankylostomes in the general population twenty of the heaviest infested of these 275 persons have been considered separately in Table XVI of the Appendix Except in a single instance

where the Ankylostomes are nearly equal to the number of Necators the Necators are seen to far outnumber the Ankylostomes We have —

ANKYLOSTOMES		NECATORS		TOTAL		GRAND TOTAL
Male	Female	Male	Female	Male	Female	
80	130	1 931	1 760	2 011	1 890	3 901

The percentage of Ankylostomes to total worms in this case is —

(a) 7 per cent (ratio female to female)

(b) 4 per cent (ratio male to male) and

(c) 5 per cent (ratio Ankylostomes total to total worms)

These percentages show that Necators exist in an overwhelming majority

Mhaskar (1924) reporting on 545 worm counts made at Trichinopoly jail shows that the highest average number of Ankylostomes per person was 5 9 harboured by persons from Tanjore district It has been the uniform experience of workers in this field that the percentage of Ankylostomes is low in India It may be assumed therefore that in the Madras Presidency at any rate the majority of hookworm infestations are due to Necators

Stoll and Tseng (1925) have shown that hookworm infestations in a Chinese group adversely affected the hæmoglobin content of the group to an extent indicated by a coefficient of correlation of about – 5 But in the same report they state that

it is known that mixed infections of Ankylostoma and Necator were being dealt with In over 10 000 adult worms recovered after treatment slightly over half were *A duodenale* On the other hand Gordon (1925) found no noticeable effect of Ankylostome (Necator ?) Ascaris or Trichuris infections on hæmoglobin percentage physique and general fitness mentality or the presence of albumen or casts in the urine of 114 native West Africans in spite of the fact that most of the infestations were quite heavy 12 of them having an average of more than 10 000 eggs per gramme of fæces Dr Gordon makes no distinction between *A duodenale* and *N americanus* but states Alder (1925) gives the proportion of *A duodenale* to *N americanus* in Freetown as 1 to 10

In order to verify actually if there was any association between the number of hookworms and the hæmoglobin percentages in our cases a correlation table was set up as shown below

The value of the coefficient is found to be 116768 ± 010123 which is only about three times its probable error But before coming to any conclusion it is necessary to test for linearity of relationship The correlation ratios computed for this table are 213744 ± 038817 and 127110 ± 010021 For all practical purposes therefore the table may be regarded as normal and the coefficient of correlation then indicates a negative association which is just significant when compared with its probable error

TABLE I

B_{hw}	Number of Hookworms Harboured															Total.
	0—25	25—50	50—75	75—100	100—125	125—150	150—175	175—200	200—225	225—250	250—275	275—300	300—325	325—350	350—375	
Hæmoglobin Index 35—45	2		1													3
45—55	20	2	3		1	1	1							1		9
55—65	62	19	5	3	2	2	1			1	1				1	97
65—75	68	11	9	3	1			1	[illegible]			1				98
75—85	21	6	4													33
85—95	11	1	2													14
95—105	1															1
Total	187	30	24	6	6	7	2	1	2	1	1	1		1	1	[illegible]

This suggests that there might likewise be some relationship between egg counts and hæmoglobin percentages. The correlation table set up in the same manner as the one for worm counts and hæmoglobin readings is given below:—

TABLE II

B_{he}	Number of Eggs per Gramme																		Tot
	0 to 500	500 to 1 000	1 000 to 1 500	1 500 to 2 000	2 000 to 2 500	2 500 to 3 000	3 000 to 3 500	3 500 to 4 000	4 000 to 4 500	4 500 to 5 000	5 000 to 5 500	5 500 to 6 000	6 000 to 6 500	6 500 to 7 000	7 000 to 7 500	7 500 to 8 000	8 000 to 8 500	8,500 to 9 000	
Hæmoglobin Index 35—45	1	1	1																3
45—55	10	4	2	2	2			1	1									1	2[illegible]
55—65	7	18	5	6	2	3	3	1		1		1							9
65—75	59	16	9	5		3	[illegible]			1			1	1		1			95
75—85	22	5	3	3															33
85—95	12	1					1												14
95—105	1																		1
Total	168	45	20	16	4	6	6	2	1	2		1	1	1		1		1	275

e coefficient of correlation is – 1203042 ± 040086 and the correlation are 2386909 ± 038357 and 1158408 ± 039810 respectively These ents indicate an association between egg counts and hæmoglobin that is gnificant, as was the case between worm counts and hæmoglobin hat the coefficients of correlation between eggs per gramme and hæmoglobin between worm counts and hæmoglobin should be identical suggests the ence of a close relationship between egg counts and worm counts The lation table set up for egg counts and worm counts is shown below —

TABLE III

Number of Hook[worms]	Number of Eggs per Gramme																		Total
	0 to 500	500 to 1 000	1 000 to 1 500	1 500 to 2 000	2 000 to 2 500	2 500 to 3 000	3 000 to 3 500	3 500 to 4 000	4 000 to 4 500	4 500 to 5 000	5 000 to 5 500	5 500 to 6 000	6 000 to 6 500	6 500 to 7 000	7 000 to 7 500	7 500 to 8 000	8 000 to 8 500	8 500 to 9 000	
0—25	154	23	8	1			1												187
25—50	9	17	7	3	1	1	1												39
50—75	4	4	4	9			[illegible]		1										24
75—100						2	1						1						6
100—125	1	1	1		1	1												1	6
125—150					1	1	1												3
150—175								1		1									2
175—200						1													1
200—225										1						1			2
225—250								1											1
250—275				1															1
275—300														1					1
300—325																			
325—350					1														1
350—375												1							1
Total	168	45	20	16	4	6	6	2	1	2		1	1	1		1		1	275

The appearance of the table itself suggests a close association The coefficient of correlation is + 713026 ± 018219 which is very highly significant It is possible to suppose that when the number of observations is large this coefficient will approach unity This means that when worm counts are known it is possible on the average, to estimate the egg counts and vice versa The correlation ratios 725622 ± 019259 and 520652 ± 029619, indicate the normal nature of the association that exists between worm counts and egg counts

The regression equation giving the average eggs per gramme (x) for persons whose degree of hookworm infestation is known is in the usual notation —

$$(x-\text{mean})-r\,\frac{\sigma x}{\sigma y}\,(y-\text{mean})$$

$$\text{or,}\quad (x-838)=\frac{7130\times 1191}{19}\times(y-31)$$

$$\text{or}\quad x=18\ 1049\,y+222\ 4$$

$$\text{or approximately,}\quad x=18\,y+222$$

This is an important relationship for egg count and worm count data and it would not be wrong to suppose that the equation approximately represents the true relationship It follows that as y increases the ratio x/y slowly decreases which means that as the number of hookworms increases the egg output per worm decreases (*see* Table XV of the Appendix)

In order to see how the values of these coefficients vary for dichotomic distributions four fold correlation tables were set up in each case as follows —

TABLE IV

Bho	Below 2 000 eggs per gramme	Above 2 000 eggs per gramme	TOTAL
Hæmoglobin			
Above 55 per cent	222	21	243
Below 55 per cent	27	5	3
TOTAL	249	26	275

TABLE V

B_{hw}	Below 50 worms	Above 50 worms	TOTAL
Hæmoglobin			
Above 55 per cent	202	41	243
Below 55 per cent	24	8	32
TOTAL	226	49	275

The coefficients between counts of more than 2 000 eggs per gramme and hæmoglobin indices of less than 55 per cent and between worm counts of more than 50 per person and hæmoglobin indices of less than 55 per cent were + 188820 ± 112961 and + 150976 ± 096276 respectively These four fold coefficients which are not significant as compared with their probable errors confirm the inference from the correlation coefficients previously described that an increase in the eggs per gramme is associated with slight reductions in the hæmoglobin index The correctness of this inference is made clear by the four fold coefficient for B_{we} namely + 877798 ± 036356—showing a very high relationship between egg counts and worm counts

TABLE VI

B_{we}	Below 2 000 eggs per gramme	Above 2 000 eggs per gramme	TOTAL
Worms			
Below 50	222	4	226
Above 50	27	22	49
TOTAL	249	26	275

The importance of this negative association between egg counts and hæmoglobin necessitates its verification on a larger number of cases For this purpose the 13,303 cases (Group A_{1-3}) examined at Mandapam Camp were set up in a correlation table Bearing in mind that the number (13 303) might influence the result to some extent in the matter of probable errors etc two big random samples (Groups A and A) were taken from this parent group and treated separately as shown in Tables VIII and IX of the Appendix

TABLE VII

	A 21	Number of Eggs per Gramme																					Total
		0 to 1 000	1 000 to 2 000	2 000 to 3 000	3 000 to 4 000	4 000 to 5 000	5 000 to 6 000	6 000 to 7 000	7 000 to 8 000	8 000 to 9 000	9 000 to 10 000	10 000 to 11 000	11 000 to 12 000	12 000 to 13 000	13 000 to 14 000	14 000 to 15 000	15 000 to 16 000	16 000 to 17 000	18 000 to 19 000	21 000 to 22 000	22 000 to 23,000	27 000 to 28 000	
Hæmoglobin Index	15—25	15	16	2	5	2	2	1									1				1		45
	25—35	26	107	18	13	6	1		3				1										175
	35—45	98	311	43	4	4	4	5	1	1	1	1	0			1							494
	45—55	402	446	83	28	11	4	5	3	1		1	1	1				1		1			1 078
	55—65	3 369	960	211	73	18	21	12	•	10	[illegible]	1	1		4		1		1				4 686
	65—75	3 671	903	224	93	31	13	11	6	7			1		1		1						4 962
	75—85	1 760	232	88	46	27	11	7	5	6	1			1				1				1	1 686
	85—95	110	24	21	12	5	3	1					•	1									177
Total		9 041	2 999	690	294	104	59	42	20	25	4	3	4	3	[illegible]	1	3	2	1	1	1	1	13 303

Four fold correlation tables (*see* Tables X, XI and XII in Appendix) were also set up as before, and the different coefficients are given in the following table The coefficients for groups B_{he} and B_{hw} are also included for easy reference —

Coefficients	A_{123}	A_1	A_2	B_{he}	B_{hw}
γ	-1322 ± 0057	-1276 ± 0099	-1879 ± 0101	-1203 ± 0401	-1168 ± 0401
η_x	2649 ± 0054	1751 ± 0097	2445 ± 0104	2387 ± 0383	$2137 \pm {\cdot}0389$
η_v	2022 ± 0056	2015 ± 0096	2918 ± 0095	1458 ± 0398	1271 ± 0400
γ_t	$+1983 \pm 0156$	$+3062 \pm 0253$	$+4245 \pm 0259$	$+1888 \pm 1130$	$+1510 \pm 0963$

It is interesting to note that all these coefficients taken with their signs confirm the inferences previously drawn and they all point to a single result namely, that there is a slight inverse association between hæmoglobin percentage and either worm count or egg count

The values of the means, standard deviations and coefficients of variation for these groups are collected in the following table for easy reference —

Groups	Mean	Standard deviation	Coefficient of variation
A_{123}	$1023 \pm 6\ 5$	$1118 \pm 4\ 6$	$109\ 3 \pm 0\ 83$
A_1	$1153 \pm 11\ 5$	$1147 \pm 8\ 1$	$99\ 5 \pm 1\ 22$
A_2	$1099 \pm 13\ 2$	$1265 \pm 9\ 3$	$115\ 2 \pm 1\ 62$
B_{he}	$838 \pm 48\ 6$	$1194 \pm 34\ 3$	$142\ 5 \pm 9\ 50$
B_{hw} (hookworms)	34 ± 2	$49 \pm 1\ 4$	$114\ 1 \pm 9\ 41$

The mean egg counts for hæmoglobin readings at stated intervals for each group are :—

Groups	Mean Egg-counts for Hæmoglobin Readings					
	Greater than 55 per cent	Less than 55 per cent	Greater than 65 per cent	Less than 65 per cent	Greater than 75 per cent	Less than 75 per cent
A_{n}	947	1,151	951	1,099	869	1 065
A_1	1 091	1,744	1,088	1,233	1,114	1,161
A_2	1 014	1,849	991	1,237	1,046	1 111
B_h	708	1,141	799	913	532	902
B_{hw} (hookworms)	33	42	30	38	23	37

The quartiles were also computed to describe the data in regard to the intensity of infection They are —

Groups	Eggs per Gramme			Percentage of Hæmoglobin Quartiles		
	1st Quartile	2nd Quartile	3rd Quartile	1st	2nd	3rd
A_{132}	555	820	1,516	62	68	73
A	656	906	1,728	63	69	73
A_2	600	850	1,589	63	69	74
B_{ho}	318	444	967	62	69	74
B_{hw} (worms)	9	18	37	62	69	74

These statistical constants show conclusively not only that the samples and the parent group are homogeneous and alike in properties but also uniformly show a progressive decrease in egg counts with increase in hæmoglobin content

Details regarding the intensity of infestation and the limits of hæmoglobin content in the majority of the population are shown by the quartiles For instance, we infer that more than three fourths of these people have hæmoglobin readings above 60 per cent indicating, in a way, that these necator infestations are not injurious to any great degree as far as hæmoglobin is concerned The quartiles

for egg counts similarly show that Necator infestations in these groups are not heavy, as three fourths have egg counts of less than about 1 500 in Group A and less than 1 000 in Group B Furthermore in Group B it is important to observe that more than three fourths have less than about 40 worms per person The median values for Group B shown under 2nd quartiles indicate that nearly half the people have less than 18 worms each Median egg counts do not exceed about 800 to 900 in Group A and about 400 to 500 in Group B All these appear to confirm the inference that Necator infestation in the sets of data studied is not perceptibly harmful in the sense that Ankylostome infestations will be shown to be

In contrast to the effect of Necator infestations as anæmia producing agents data on 18 persons experimentally infected with Ankylostomes are presented in Tables XIII and XIV of the Appendix In Table XIII the details are given for ten of these persons who have been treated until cured The other eight are still under observation The data on these will be found in Table XIV

The length of time the infection was harboured by those listed in Table XIII was not less than three months and not more than 11 months the average being about seven months The fluctuations of the hæmoglobin in these cases form a remarkably interesting study During the period of the infection the hæmoglobin of case No 1 decreased by 38 per cent case No 2 by 20 per cent case No 3 by 41 per cent case No 4 by 13 per cent No 5 by 50 per cent No 6 by 67 per cent No 7 by 50 per cent No 8 by 50 per cent No 9 by 53 per cent and No 10 by 0 per cent Thus in only one case (case No 10) was there no decrease of hæmoglobin and this person harboured only two Ankylostomes The decrease varied from 20 to 60 per cent the large losses corresponding to the 7 to 11 months periods of harbouring infections That the decrease in hæmoglobin was due to hookworm infection is demonstrated by the increases in hæmoglobin a few months after treatment Cases Nos 1 2 and 3 began to show the ill effects of the infection soon after they were experimentally infected and each of them was given tonics and special diet during the infection and for a long time after they had been cured Cases Nos 6 to 9 have just been cured and no change in their hæmoglobin readings have been detected

The worm counts on these ten cases were very carefully done and the table shows that practically every worm recovered belonged to the species *A duodenale* In only two cases were 12 and 2 Necators found in company with 70 and 97 Ankylostomes respectively It may fairly be stated therefore that the extraordinary decrease in the hæmoglobin was due solely to infestation with *Ankylostoma duodenale* and the subsequent rise to their complete absence It may be noticed also that the large decreases in hæmoglobin in cases Nos 6 to 9 were due to the relatively larger number of Ankylostomes harboured by them These results confirm the generally recognized fact that infestations with *A duodenale* are far more serious than equal infestations with *N americanus* The results also lend support to the assumption on our part that infestations in southern India are chiefly

due to *N americanus* for no such reductions of hæmoglobin were found among the twenty heaviest infested of the 275 persons in Group B as Table XVI in the Appendix shows

Up to this point the analyses of the data in hand indicate but a slight negative association between the size of egg counts or worm counts and the percentage of hæmoglobin in the infested groups The question now arises as to whether there is an upper limit of tolerance to Necator infestations and if so at what point this begins Consider the mean egg counts corresponding to increasing intervals of hæmoglobin percentages

Percentage hæmoglobin	A [illegible]	A	A_2	B_{he}	B_{hw} (worms)
15—25	2 633	3 375	6 200		
25—35	1 889	2 250	2 889		
35—45	1 080	2 233	2 239	750	29
45—55	1 325	1 493	1 449	1 181	44
55—65	941	1 096	1 051	82	37
65—75	907	1 0 9	964	888	34
75—85				553	23
85—95				500	21
95—100				2 0	13

The gradual decrease in egg counts with rise in hæmoglobin suggested the computation of the mean egg counts for persons having hæmoglobin readings of more than 55, 65 and 75 per cent respectively This gives

	A_2	B_{he}	B_{hw} (worms)
Greater than 55 per cent	947	798	33
65	951	799	30
75	869	532	23

The mean eggs per gramme or worms per person for more than 55 and 65 per cent of hæmoglobin being practically stationary, the large drop in the corresponding mean values for more than 75 per cent hæmoglobin indicates that Necator infestations averaging up to about 25 to 30 worms or producing counts of 700 to 900 eggs per gramme would produce no measurable reduction in the hæmoglobin

The mean values of the hæmoglobin content for more than and less than 2,000 eggs per gramme, as well as for less than 7,000 and more than 7,000 eggs per gramme, and in the case of B_{hw} for less than and more than 100 worms and less than 200 and more than 200 worms are calculated and given in the following table —

Groups	Total population	Less than 2,000 E P G	Less than 7,000 E P G	Total population	Greater than 2 000 E P G	Greater than 7 000 E P G
A_{123}	65	65 ± 09	65 ± 09	65	63 ± 39	61 ± 1 78
A_1	66	66 ± 14	66 ± 14	66	64 ± 52	59 ± 2 55
A_2	66	67 ± 15	66 ± 15	66	61 ± 70	61 ± 3 04
B_{he}	66	67 ± 64	66 ± 62	66	63 ± 1 66	64 ± 2 54
B_{hw} (worms)	66	67 ± 63 Less than 100	67 ± 62 Less than 200	66	62 ± 1 59 Greater than 100	63 ± 2 44 Greater than 200

It is clear from this table that mean values of the hæmoglobin of less than 2,000 eggs per gramme and of less than 7 000 eggs per gramme are practically the same, whereas the means for more than 7 000 eggs per gramme are slightly smaller than those for more than 2 000 eggs per gramme Hence it is possible that Necator infestations showing average counts between 2,000 to 7 000 eggs per gramme would produce a measurable decrease in the hæmoglobin, but that a definite break in the compensatory powers of the individual, giving rise to real hookworm disease, would only accompany infestations with egg counts exceeding an average of 7,000 eggs per gramme

Discussion

A study of the data presented in these two groups shows that we were dealing with very light infestations almost entirely Evidence was put forward also to show that the infestations consisted chiefly of *N. americanus*, and that probably

not more than five per cent of the worms belonged to the species of *A duodenale* To demonstrate the striking difference between the effect of *A duodenale* and *N americanus* as anæmia producers the results of observations on 18 persons experimentally infected with the former species were cited These compared with the twenty heaviest infested of Group B show clearly that Ankylostome infections are much the more important of the two This has been a recognized fact generally for some years but probably nowhere has it been more clearly demonstrated

Aside from the actual intensities of the infestations dealt with here and those studied by Stoll and Tseng (1925) in China, it is possible that the higher coefficient of correlation obtained by them was largely due to the fact that the proportion of Ankylostomes in the Chinese group was far greater than in the Indian groups By analogy it is possible to conceive that if 50 per cent of the worms in the groups studied here had been Ankylostomes, the coefficient of correlation would have been much more highly significant Gordon (1925) was unable to demonstrate any reduction of hæmoglobin among 114 native West Africans despite the fact that 12 of them had egg counts of more than 10,000 eggs per gramme Here again it appears that not more than one tenth of the parasites harboured were *Ankylostoma duodenale*

None of the coefficients of correlation between egg counts and hæmoglobin or between worm counts and hæmoglobin for the Indian groups were high, but the fact that the coefficients were uniform and almost identical for all the groups indicates conclusively that there is a certain amount of association even with these mild infestations between egg counts and hæmoglobins

The average number of eggs per gramme per female worm on the formed basis computed from the mean is 49 2 Computed from the median the factor becomes 49 3 Hence Stoll's factor for the Madras Presidency would work out at about 49 eggs per gramme per female Necator on the formed basis That there must be some such fixed relationship between eggs per gramme and female worms is clear from the highly significant correlation indicated by the coefficient + 743026 ± 018219 between the egg counts and worm counts on Group B, although it is possible that the deviation of this coefficient from unity is due to such specific factors as mixed Ankylostome and Necator infections or variations in the egg output per female as the number of worms in the intestines increases or decreases Both the regression equation and the four fold correlation value suggest the possibility of the number (49) being reduced as the infestation becomes heavy

CONCLUSIONS

(1) There is an overwhelming preponderance of *N americanus* over *A duodenale* in southern India

(2) Hookworm infestations in this area are generally light the average nowhere exceeding 2 000 eggs per gramme

(3) The average number of eggs per gramme per *female* (*formed basis*) works out at about 50

(4) *The correlation between egg counts and worm counts is highly significant* the coefficient being + 7430?6 ± 018?19

(5) The *association between egg-counts and hæmoglobin is about* — 12 showing a slight negative relationship

(6) The association between worm counts and hæmoglobin is also approximately equal to — 12 indicating that any effect on the hæmoglobin could be detected equally as well by egg counts as by worm counts

(7) It may be stated that no effect on the hæmoglobin is likely to be detected with average counts of about 900 eggs per gramme or with average infestations of 30 worms per person With egg counts between ? 000 and 7 000 the effect becomes measurable while average counts of more than 7 000 eggs per gramme will usually be associated with definite hookworm disease

REFERENCES

GORDON I M (19?5) The Effect of Ankylostome Ascaris and Trichuris Infections on the Health of the West African Native *Ann Trop Med and Parasit* Vol XIX No 4 pp 4?9—460

MHASKAR K S (19?4) *Ind Med Res Memoir* No 1 pp 1—9?

STOLL N R (19?3) An Effective Method of Counting Hookworm Eggs in Fæces *Amer Jour Hyg* Vol III, No 1 pp 59 70

Idem (19 4) The Significance of Egg Count Data in *Necator americanus* Infections *Ibid* Vol IV No 5 pp 466 500

Idem with TSENG HSIEN WU (19?5)

TABLE X.

A_{133}	Below 2 000 eggs per gramme	Above 2 000 eggs per gramme	TOTAL
Above 55 per cent	10 529	982	11 511
Below 55 per cent	1 511	281	1 792
TOTAL	12 040	1 263	13 303

TABLE XI

A_1	Below 2 000 eggs per gramme	Above 2 000 eggs per gramme	TOTAL
Above 55 per cent	3 585	508	4 093
Below 55 per cent	307	124	431
TOTAL	3 892	632	4 524

TABLE XII

A_2	Below 2 000 eggs per gramme	Above 2 000 eggs per gramme	TOTAL
Above 55 per cent	3 434	318	3 752
Below 55 per cent	305	125	430
TOTAL	3 739	443	4 182

TABLE XIII.

Showing the number and species of worms recovered from ten of the experimentally infected persons, the loss of hæmoglobin and weight and the increase of hæmoglobin from 6 to 7 months after treatment

No	Date infected	Hæmoglobin			Loss of weight	Length of time infection was harboured	Worms recovered				Total
		On date of infection	On date treated	6 to 7 months after treatment	Pounds	Months	Ankylostomes Males	Ankylostomes Females	Necators Males	Necators Females	
1	1-6-1926	80	50	80		5	31	39	6	6	82
2	1-6-1926 / 7-9-1926	75	60	90	6½	5	47	50		2	99
3	17-5-1926 / 7-9-1926	85	50	85	10	4	82	109			191
4	17-5-1926 / 7-9-1926	80	70	Discharged	4	3½	30	42			72
5	17-5-1926 / 7-9-1926	80	40	60	13	7	61	102			163
6	17-5-1926 / 7-9-1926	90	30	*	14	11	243	265			508
7	17-5-1926 / 7-9-1926	70	35	*	8	11	131	113			244
8	17-5-1926 / 7-9-1926	85	40	*	9	11	196	170			366
9	17-5-1926 / 7-9-1926	75	35	*	15	11	131	129			260
10	17-5-1926	80	80	Discharged		3½	1	1			2

Note —Nos 1 2 and 3 were given extra diet and tonic both before and after treatment

* Nos 6 to 9 have just been treated and no record of improvement of hæmoglobin is available

TABLE XIV

Showing the egg counts, loss of hæmoglobin and loss of weight of eight of the experimentally infected persons approximately one year from date of infection

No	Date infected	Hæmoglobin		Maximum loss of weight Pounds	Egg-count per gramme on July 31st 1927
		On date infected	On July 31st, 1927		
1	1-6-1926 7-9-1926	80	40	7	5 900
2	1-6-1926	90	75	5	2 200
3	17-5-1926	80	80	4	4 200
4	17-5-1926	80	80		1 200
5	17-5-1926	85	75	2	2,200
6	17-5-1926 7-9-1926	90	65	10	9 800
7	17-5-1926 7-9-1926	80	60	5	8,200
8	17-5-1926 7-9-1926	90	70	5	2,500

Note—These persons are still under observation

TABLE XV.

Giving further details of the experimentally infected persons shown in Table XIII These counts are on the 'unformed basis'

No	First Day			Second Day			Third Day			Total eggs	Average eggs per day	Number of female worms	Average eggs per day per female worm
	Weight of stool in grammes	Eggs per gramme	Eggs per day	Weight of stool in grammes.	Eggs per gramme	Eggs per day	Weight of stool in grammes	Eggs per gramme	Eggs per day				
1	220	2 550	561 000	187½	[illegible] 100	351 750	148	2 150	318 200	1 230 950	410 316	45	9 118
2	241	8 950	2 156 950	141½	5 150	744 175	133½	7 700	1 027,950	3 9[illegible]9 075	1 309 691	52	25 186
3	4[illegible]6	5 700	2 426 200	554	6 000	3 324 000	134	9 000	1 206 000	6 958 200	2 319 400	109	21 279
4	294	4 3[illegible]0	1 278 900	286½	6 050	1 733 325	147	6 700	984 900	3 997 125	1 332 375	42	31 725
5	307	6 700	2 056 900	507	3 0[illegible]0	1 548 350	435	3 400	1 479 000	[illegible]082 250	1 694 085	102	16 609
6	464	9 000	4 176 000	26	28 600	743 600	135	21 300	2 8[illegible]5 500	7 795 100	2 598 366	265	9 805
7		Average eggs per gramme			5 100							113	
8	78	22 800	1 778 400	156	18 400	2 870 400	231	10 300	2 379 300	7 028 100	2 342 700	170	13 780
9	174	15 100	2 679 000	452	9 300	4 203 600	226	11 000	2,486 000	9 369 200	3 123 066	129	24 210
10	484	100	48 400	284	100	28 400	272	50	13 600	90 400	30 133	1	30 133

Note —Collection of total daily output on No 7 was not made The count represents the average of two counts on each of five successive days

tube may be taken as safe when the microscopists doing the counts are experienced men [(*see* also Chandler (2)] Each fæcal specimen used was examined by the Stoll method and then *if it showed no hookworm eggs by the Willis* method of flotation in salt solution(3) All persons examined were included in obtaining the average egg counts those showing no hookworm eggs by the Stoll method and those negative to the Willis method being given a zero egg count It would seem better in the light of later experience to include persons showing negative egg counts positive Willis tests as giving an arbitrary count of 50 or 100 eggs per gramme

All of the stools used for counting were classed as mushy, under Stoll s classification but are reported in this paper on the 'basis formed fæces' that is double the actual count made As a factor by which these egg counts may be expressed in numbers of worms Stoll s figure of 11 eggs per gramme per female worm may be used(1*b*) For the approximate total number of worms of both sexes the average egg counts per gramme may be divided by 22 In a series of worm counts made in Ceylon in 1925 all the worms found were of the species *Necator americanus*(4)

Quantitative and Qualitative Estimates of Hookworm Infection in Ceylon

Table I gives the results by provinces of the survey of Ceylon As has been stated 32 507 persons were examined The qualitative information obtained showed that 90 5 per cent of these people were infected with hookworms varying from 84 9 per cent infected among the 3 575 residents of Uva *Province* to 96 5 per cent of 1 301 persons from the north central province These infection rates were all high and gave little information of value in determining variations in the actual hookworm conditions of the various parts of the island On the basis of these results the only information obtainable in bulk before the publication of egg counting methods the hookworm problem of Ceylon would have been regarded as uniform throughout the island and active control measures would have necessitated equal emphasis and expenditure in *all* provinces The *information obtained* from the egg counting technique however gave another opportunity for evaluating the problem

The 32 507 persons examined had an average hookworm egg count per gramme 'basis formed fæces' of 2 200 This average varied from 1 600 eggs per gramme per person in the Uva Province to 3 100 in the north western and north central provinces where 3 575 3 408 and 1 301 persons were examined respectively The intensities of hookworm *infection in the nine provinces of Ceylon* (it is not feasible here to go into smaller divisions) fell into three groups The north central and north western provinces had an average infection per person of 3 100 eggs per gramme the northern eastern and Sabaragamuwa Provinces 2 300 to 2 700 e p g the Uva central southern and western provinces *1 600 to* 1 900 e p g

TABLE I

Hookworm Infection and Intensity Rates for the Provinces of Ceylon

Provinces	Number of persons examined	Average egg count per gramme per person basis formed fæces	Percentage infected with hookworms
Northern	[illegible] 4[illegible]6	[illegible] 700	95 2
North Central	1 301	3 100	96 5
North Western	3 408	3 100	96 0
Eastern	3 396	2 300	91 4
Uva	3 575	1 600	84 9
Southern	3 207	1 900	90 7
Central	7 363	1 900	89 3
Sabaragamuwa	3 879	2 400	90 0
Western	3 902	1 900	88 1
All Provinces	3[illegible] 507	2 [illegible]00	90 5

These three groups of provinces did not correspond to any geographical rainfall occupational or other classification The most lightly infected group contained the provinces with the heaviest rainfall the largest population and the greatest area in cocoanut rice rubber and tea plantations with the exception of Sabaragamuwa which belonged in this group under these classifications but was in the more heavily infected hookworm group The provinces with the lightest rainfall and the smallest density of population were in the two more heavily infected groups In these provinces the population lives in scattered villages usually at the foot of the bunds of large tanks from which their rice and other fields are irrigated Rainfall is confined largely to one monsoon covering about three months or less in the year

Most of the hookworm control work of the past ten years was confined to the provinces which gave the lowest intensity rates None of the persons examined in the four dry provinces gave histories of previous treatment for hookworm infection

nor had any extensive control work been done in those provinces The examination in the southern and western provinces were made of untreated people only but the control campaigns in their areas might have operated to reduce their infections Of the 14 817 persons examined from the central Uva and Sabaragamuwa provinces 16 *per cent had received previous treatment Those previously treated* regardless of the date of such treatment had an average of 1 200 e p g , as against 2 100 e p g for those not giving histories of previous treatment A more detailed discussion of the effect of treatment and of hookworm re infection in these areas was published some time ago(5)

It is impossible to say what the pre hookworm campaign distribution of infection was but at the period of survey there was no relation between rainfall and hookworm intensity with the exception of certain local areas of the southern province The presence of tanks and the situation of the villages had apparently minimized the effect of rainfall if there was any such relation originally It is possible that the rainfall of the southern provinces was excessive and that too much rain may be as detrimental *to the development of a heavy average* hookworm infection as is too little rainfall in a country without tanks and irrigation

The Intensity of Hookworm Infection in Ceylon as related to Age

The results of this survey of Ceylon as far as they concerned the intensity of hookworm infection in relation to age and sex were published in an article dealing with the correction of hookworm rates(6) There was no significant variation between the average egg counts of males and females in any age group the greatest difference being only 200 eggs per gramme in the two to six years group where the males had the higher figure The largest difference in percentage of infection was in the 51 years and over age group where 93 per cent of the males were infected and 96 5 per cent of the females Children below two years of age were not examined but children of both sexes between the ages of two and six inclusive had an average infection of 1 500 eggs per gramme and 75 5 per cent of the 3 209 examined were *found infected The average infection of all persons seven* years old and over was 2 300 e p g and in none of the six age groups used was there any significant variation from this average Children from seven to ten years of age had an average *infection of 2 300 e p g a rate which was constant throughout* the other age groups

This would seem to indicate that the adult level of infection in Ceylon was attained by children of the younger ages There *is however one factor* which must be considered before any such conclusion should be stated * The number of hookworm eggs per gramme is known to vary with the type of stool being higher in formed stools than in mushy *If the female hookworm lays her eggs at a* fairly constant rate which seems to be the case the variations in eggs per gramme for types of stools would seem to be due to the dilution of the eggs in the fæcal mass

* Suggestion made to writer by Stoll

the larger stool giving the lower egg count per gramme The relative sizes of child ren s and adult s stools have apparently not been studied but it seems reasonable to suppose that children s stools are smaller than those of adults an l that conse quently an identical egg count per gramme m ght connote an entirely different number of female worms in the child than in the adult This remains to be proved but meanwhile one cannot draw any conclusions as to numbers of worms represented from egg per gramme data in children In spite of these considerations the fact remains that numerically the egg counts per gramme of school children from 7 to 15 years were the same as those of ad ilts and that consequently examinations of the school children in Ceylon will give a result in eggs per gramme which can be regarded as applying to the a l ılts This fact would seem to be true also in Mexico where from figures published by Carr(7) it is shown that examination of children from 6 to 15 years of age gives a figure for average eggs per gramme slightly higher than that found amongst persons 16 years old an l over Such relations may however be changed by control work more particularly by mass treatment of one group of a populat on school chil lren for instance

TABLE II

Pe cer tages of Persons i Various Egg Co ı t Croups Nortl Western an l Sab iragu nuu ı Prov nces

Age roup	Numbe of persons exam ed	Not infec ted with ookworm		1—5ʋ9 e p g		600—1 799 e p g		1 800—3 3ʋ9 e p g		3 400—10 9ʋ e p g		10 800 and over e p g	
		No	Per cent	No	Per cent	No	Per cent	No	Per cent	No	Per cent	No	Per cent
— 6	7[illegible]	17	3	101	13	[illegible]35	31	0	[illegible]6	38	[illegible]	17	
7—10	1 5 3	68	5	14	[illegible]	4[illegible]	[illegible]	6 7	41	138		53	3
11—14	1 07	47	4	7	[illegible]	3 7	31	456	43	114	1	31	3
15—18	667	4	6	69	10	169	5	88	44	8	1	1	3
19—40	[illegible] 591	149	6	64	1	751	[illegible]	1 0 3	4	[illegible] 7	10	77	3
41—[illegible]	3	1	6	3	10	106	[illegible]9	15	41	37	10	14	4
51 and over	177	8	5	1[illegible]	8	[illegible]9	33	77	44	9	5	9	5
Al. ages	16	5[illegible]7	7	7 3	10	[illegible] 14	30	[illegible] 895	41	671	9	[illegible]2[illegible]	3

The results of examinations made in two provinces north western and Sabaragamuwa were further classified into egg count groups so as to determine the percentages of the population in various intensity ratings Since sex had been shown to make little difference in average egg counts it was not considered in the classification The average infection of the 7 160 persons included in Table II was 2 800 e p g

The essential similarity numerically of the distribution of hookworm infection intensities in all age groups with the exception of the youngest is well shown in Table II Of the children between two and six years of age inclusive 31 per cent had infections with counts of between 600 and 1 800 eggs per gramme 26 per cent had counts between 1 800 and 3 400 e p g, and 23 per cent showed no infection by either the Willis or Stoll methods For all persons examined of all ages 41 per cent had egg counts between 1 800 and 3 400 e p g 12 per cent were above this group 7 per cent were not found infected and the remainder 40 per cent had counts between 1 and 1 800 e p g It would seem that in these two provinces where the average infection was approximately 120 hookworms per person and the infection rate was 93 per cent only 53 per cent of the population had more than approximately 80 worms per person Smillie and Augustine and Smillie and Spencer have stated that intensities of infection of 100 hookworms per person and up should be regarded as true hookworm disease and that an infection of less than 25 worms should be classed as a purely carrier state(8 and 9) In these two provinces then instead of considering the hookworm problem as one in which 93 per cent of the people were infected we may say that but 53 per cent had hookworm disease the remaining 47 per cent being either not infected or so lightly infected as not to suffer any measurable diminution in health In other provinces of Ceylon in which the average intensity of infection was lower the occurrence of actual cases of hookworm disease would be in a *still* lower ratio

Hookworm Infection and Occupation

A group of 1 822 males 15 years of age and over residents of the southern and north western provinces were classified as to occupation The results of this classification are given in Table III For comparison the average egg-counts of children from 2 to 14 years and of females 15 years and over in the same areas are also given

The average egg count per gramme for the 5 595 persons examined in *these areas* was 2 700 a figure which also represents the count found in children under 14 and in females over that age The average counts in males over 14 varied from *1 800* e p g in 73 professional men to 3 600 in 293 cultivators Traders students and professional men were below the average count of the group they were all mainly town residents and wore shoes to some extent the professional *men—doctors* lawyers etc —probably being most constant in this regard Coolies included all government and estate labourers mostly Indian Tamils and had an average of 3 000 e p g

TABLE III

Hookworm Infection and Occupation, Southern and Part of North Western Provinces

Age, sex, and occupation	Number of persons examined	Average e p g per person * basis formed	Percentage infected with hookworms
Males and females 2 to 14 years	2 537	2 600	90 6
Males only 15 years and over			
Coolies	931	3 000	93 1
Traders	366	2 300	89 3
Cultivators	293	3 600	98 6
Students	111	2 200	91 0
Professional	73	1 800	72 6
Others	48	2 800	83 3
All males—15 years and over	1 822	2 800	92 0
Females—15 years and over	1,166	2 800	95 9
Total for area	5 523	2 700	92 1

Figures were available for men working in various forms of agriculture in two areas of the southern province and were of some interest In these areas the average intensity of infection was lower than that found in the area considered in Table III just mentioned so the average of infection of the cultivators was not so high, but the relative intensities of infection were worth noting

Table IV gives the average infections of male adults engaged solely in various forms of cultivation and gives the same figures for the remainder of the population of the two areas

TABLE IV

Hookworm Intensities of Male Adults engaged in Agricultural Occupations Two Districts of Southern Province

Male adults engaged solely in	Number of persons examined	Average egg count per gramme per person based formed
Rice fields	1[illegible]	[illegible] 580
Tea estates	100	[illegible]400
Cocoanut estates	100	2 000
Rubber estates	100	1 900
Remainder of population	1 8[illegible]4	[illegible] 000

In the Galle and Matara areas of the southern province the average infections of male adults engaged in rice and tea cultivation were significantly higher than those found in men engaged in cocoanut and rubber estate work and than those of the remainder of the population the rate for rice field workers being the highest The cultivators of Table III were mainly engaged in rice and cocoanut cultivation since the great majority of labourers on tea and rubber estates were Indian Tamils and were classified as coolies It will be seen that the cultivators of Table III had an average infection some 33 per cent higher than that of the general population and that the rice field workers of Table IV were 10 per cent above the general level of infection while the workers on cocoanut estates were not more heavily infected It would appear then that rice cultivation in Ceylon was in some way associated with a hookworm infection about 35 per cent higher than that found in the general population This is difficult to explain The use of night soil as fertilizer is by no means customary in Ceylon and even in a country where it is so used extensively the China Hookworm Commission(10) could find no indication that it resulted in heavy infections in rice field workers Other findings of this survey seem to point to the surroundings of the living quarters as the source of the greater part of the hookworm infection of Ceylon so it is possible that further study of the situation as to the amount of moisture character of soil etc , around the homes of rice cultivators would reveal the sources of their infection more certainly

Conclusions

Hookworm infection in Ceylon was found to be widespread, about 90 per cent of the population being infected The incidence of the infection was somewhat higher in the provinces in the dry zone where tanks and irrigation are common and rainfall is confined to a short period The wet zone of the island has had some ten years of hookworm control work which may have resulted in the lower rates but it was suggested that frequent and heavy rains were also a deterrent influence

Quantitative estimations of this widespread infection showed that the average Ceylonese had an intensity rate of only 2 200 eggs per gramme basis formed fæces' approximately 100 hookworms As compared with results reported from Central America this was a rather low grade of infection The long dry season of the north the excessive rainfall of parts of the south and the results of hookworm control work were possibly responsible for this in varying proportions

The average egg counts per gramme of the children from seven to fourteen years of age were found to be numerically equal to those found amongst adults making it possible to estimate adult averages by examining school children where no control work had voided the relationship A study of the distribution of the infection in the population of two provinces in which the average rate was 2 800 eggs per gramme showed that but 53 per cent of the people had what Smillie classified as hookworm disease the remainder being merely carriers of worms As against the qualitative figure of 90 per cent infected with hookworms it was possible to reduce the problem to a conception in which slightly less than 50 per cent of the people of the island as a whole had hookworm disease

The relatively high infection in the children the habits and customs of the people the fact that night soil was not used as a fertilizer to any extent at least and the general lack of latrines made it seem clear that the hookworm infection of Ceylon was acquired from pollution around the living quarters of the people Against this fact was the relatively high intensity of infection amongst adult males engaged in rice cultivation about 35 per cent higher than that of the surrounding peoples

References

(1) Stoll, Norman R (19 3) Investigations on the Control of Hookworm Disease XV An Effective Method of Counting Hookworm Eggs in Fæces *Amer Jour Hyg* Vol III No 1 pp 59—0

(1b) *Idem* XVIII On the Relation between the number of Eggs found in Human Fæces and the number of Hookworms in the Host *Ibid* No 2 pp 156—179

(2) Chandler Asa C (19) The Measure of Hookworm Infection in Communities *Ann Trop Med and Parasit* Vol XIX No 2 pp 191—210

(3) Willis H H (1921) A Simple Levitation Method for the Detection of Hookworm Ova *Med Jour Aust*, Vol II pp 375-376.

(4) SWEET, W C (1925) .. .. Average Egg Count per Grammo per Female Hookworm in Ceylon *Jour Parasit*, Vol XII, pp 39—4?

(5) *Idem* (1925) .. .. Hookworm Re infection *Ceylon Jour Sci*, Sect. D Med Science, Vol I, Part 3 pp 129 -140

(6) *Idem* (1929) .. . Studies on Hookworm, Ascaris and Trichuris in Panama IV The Correction of Hookworm Rates for Age and Sex *Amer Jour Hyg*, Monographic Series No 9 Jan

(7) CARR HENRY P (1926) . Observations upon Hookworm Disease in Mexico *Ibid*, Vol VI, pp 42—61

(8) SMILLIE, W G and AUGUSTINE, D L (1926) Hookworm Infestation *Amer Jour Dis of Children* Vol XXXI pp 151—168

(9) SMILLIE W G and SPENCER, CASSIE R (1926) Mental Retardation in School Children infected with Hookworms *Jour Educ Psych*, May 1926 pp 314—321

(10) CORT W W GRANT, J B, STOLL, N R and TSENO, H W (1926) Researches on Hookworm in China *Amer Jour Hyg* Monographic Series, No 7, Paper LX, Rice Cultiva tion and Hookworm Infestation

THE RELATION OF ANKYLOSTOME INFESTATION TO THE PHYSICAL FEATURES OF AN AGRICULTURAL AREA IN INDIA AND TO THE SOCIAL AND ECONOMIC STATUS OF ITS POPULATION

BY

VISHNU T KORKE F R C P (Edin) D T M (L pool)
Central Research Institute Kasauli

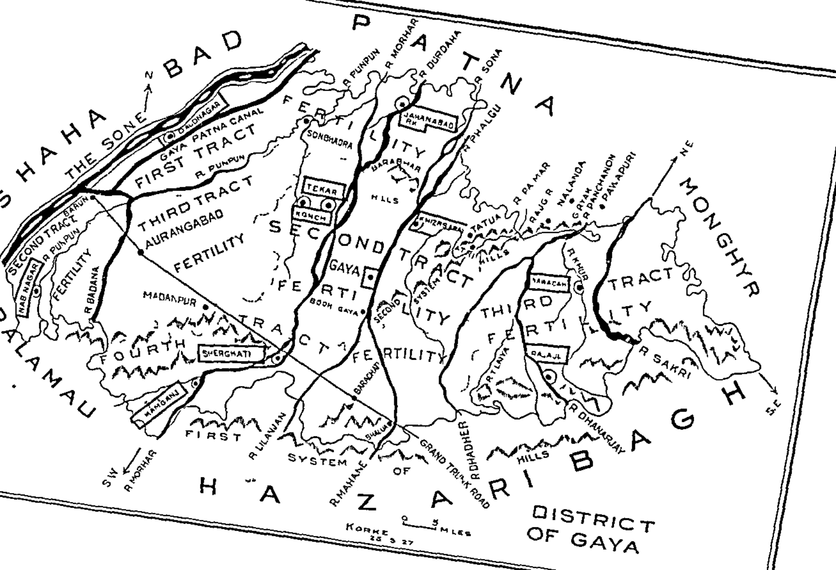
I Introduction

The observations recorded in this paper were made during the course of hookworm investigations in the Gaya district area during the months of February to April 1927 The general line of research followed has been the investigation of hookworm prevalence in relation to and the correlation of these conditions with physical features from the status of agricultural and economic conditions Such a line of research is especially important where on account of the promiscuous and insanitary habits of the agricultural population any special section is liable to be exposed to the maximum degree of parasitism

From the agricultural and economic aspects of an area one can obtain leading information regarding (*a*) the physical features of the country (*b*) the fertility of the soil (*c*) the prosperity and the growth of the agricultural family and (*d*) the period during which a peasant comes in contact with the soil The investigation also sheds an indirect light on the life cycle of hookworm in nature In this way the two aspects of an epidemiological survey viz the incidence and degree of hookworm infection in a group of population and the life history of hookworm in nature are combined into one and the investigation yields a collective result The observations were made on the agricultural group of population of the Gaya district area and the paper is illustrated by the physiographical model of Gaya

The district of Gaya (*see* Map) is essentially a rural area of about 5 000 square miles and along with the district of Patna forms a part of the right board of the Ganges The area presents some unique features in physical characters history religion and ethnology which have determined its agricultural and economic prosperity

The prosperity of the area is contributed mainly (*a*) by the hill system which rises 2 000 feet at places forms a part of the Chota Nagpur plateau and cuts up the whole edge of Gaya towards south and south east (*b*) by the twelve rivers which issue from this hill system and (*c*) by the River Sone


DISTRICT OF GAYA
SHAHABAD
PATNA
MONGHYR
PALAMAU
HAZARIBAGH
THE SONE
GAYA PATNA CANAL
DAUDNAGAR
FIRST TRACT
THIRD TRACT
AURANGABAD
FERTILITY
SECOND TRACT
SECOND TRACT FERTILITY
FOURTH TRACT
THIRD FERTILITY
TRACT
FIRST SYSTEM OF HILLS
SONBHADRA
TEKAR
KONCH
JAHANABAD
BARABHAR
HILLS
GAYA
BODH GAYA
MADANPUR
SHERGHATI
BARACHATI
NAB NAGAR
BARUN
TATUA
NALANDA
PAWAPURI
R PUNPUN
R MORHAR
R DURDAHA
R SONA
R PHALGU
R BADANA
R LILAJAN
R MAHANE
R DHADHER
R DHANARJAY
R SAKRI
R KHUR
R PAMAR
GRAND TRUNK ROAD
N
NE
SE
SW
0 5 MILES
KORKE 25 5 27

The slope of the country is from south to north towards the Gangetic valley It is comparatively rapid six to four feet in the mile and assumes the characters of an alluvial plain towards the last 30 miles from the Ganges The physical characters divide the district into two natural divisions The northern division is a comparatively flat alluvial plain and encloses about two thirds area of the district The division presents areas of fertility of varying degrees according to the nature of the soil flatness of the country and the water available for irrigation In the highly cultivated tract the density of population is about 666 the maximum In contrast the southern division which is about one third of the total district area is a sloped surface high and barren unprotected by irrigation and is incapable of cultivation It is full of brush jungle which rapidly passes into sub montane forests and was for a long time a shelter of aboriginal tribes The density of population is 278 to 257 per square mile the minimum

The second system of hills comprises an interrupted chain which runs for a distance of 40 miles from the chief town of Gaya in a north easterly direction and breaks the surface of a level plain in the heart of the district This and the third system of hills of isolated peaks like the Barabar hills are concerned with directing the course of the rivers and are associated with the ancient history of India the Buddistic religion the cave monuments and sanctity

The rivers of Gaya almost running in a parallel course bring an amount of gravel and fine sand during the floods and raise their own level when they reach the plains They have a fitful flow may fill for a few days and be almost empty for the next fortnight The indigenous system of irrigation is perpetually modifying their course with the result that the majority of them do not reach the Ganges The River Sone runs a straight course of 100 miles and is about 400 feet above the sea level when it first touches the district and has a fall of 1 75 to 2 8 feet in a mile The river distributes water to the Gaya and neighbouring districts through the distributaries of the Sone canal system The old course of the river may still be traced through the northern portion of the district and has left areas rich in soil deposits which include tracts of the first degree of fertility

Gaya is the hottest place in the province of Bihar and Orissa and also in Bengal The average maximum temperature rises to 105° F and the humidity averages 51 per cent of saturation The average rainfall is about 45 inches but owing to the position of the district from the sea it is frequently deficient fitful and untimely The humidity during the monsoons averages 84 to 87 per cent and falls lower in September

The district is occupied by the Gangetic alluvium which possesses the following characters —(1) Loam with a small proportion of sand the soil is adapted for the cultivation of paddy and requires irrigation (2) A species of hard stiff clay opening out when dry in gaping fissures It retains moisture longer and produces the spring crop (3) Sandy the soil is deposited by diluvium from the hills and carried by the rivers It can produce fine paddy when well irrigated (4) A white soil

impregnated with carbonate of soda It is an unculturable waste especially when the impregnation is great

Cow dung is an important manure but it only finds its way to the fields in the form of ashes along with the household refuse Except when human defæcation takes place in the fields human excreta does not form a fertilizing agent

The total population is roughly well over 2 millions of which only 5 per cent live in the urban areas and the remainder congregate in about 8 000 villages Gaya has excess of females over males For the purpose of investigation I have divided the age groups into three stages viz, (1) the school going age up to 20 years (2) wage earning age 20 to 55 years, and (3) advanced age over 55 years

The village community can be classified into 4 sections —(1) Agricultural group consisting of landholding and agriculturist classes which stand higher in the social grade and as a rule do not perform manual labour but employ labourers for the purpose (2) Skilled and industrious class of cultivators which are divided into whole time and part cultivators e g herdsmen (3) Landless labour class which is indirectly supported by agriculture and may move from place to place according to the demand for labourers in other areas (4) Skilled labour or village artisan class which mostly spends its life in a village and forms a recognized part of a village community Agriculturists, when fully employed work nine months in a year

The district of Gaya has been partitioned into *tracts of fertility* a recognized nomenclature for the purposes of land value assessment of rents and wages after Grierson and Stevenson Moore (1898)

In the first tract 5 acres of land in the second 6 acres in the third 8 acres and in the fourth 13 acres of land will support a family of average size in con fort

Observations on the family and land value conditions in the tracts of different fertility lead one to conclude that favourable conditions operate towards (*a*) the increase in the size of the family (*b*) the increase of the census of landholding classes (*c*) the increase of the employment of the pure agriculturist and landless labourers and (*d*) the increase of agricultural products sufficient for the maintenance in comfort of a family of average in a minimal sized land

II Material and Technique

The material for observation was derived from the sections of agricultural population residing in the 5 type areas of the northern division and 4 type areas of the southern division of the district The population was promiscuous in defæcation and free from sanitary control

Material

Northern Division First tract of fertility —(1) Area of about 606 square miles type area investigated Jehanabad on the confluence of the Morhar and Jumna about 30 miles north of Gaya Twenty eight fæcal samples were collected from the known group and 20 from an unknown group or stray (2) Area about

287 square miles irrigated by the waters of the Sone type area investigated Daudnagar on the Sone about 72 miles north west of Gaya Known samples collected 57 stray 18

Second tract of fertility —(1) Area about 288 square miles type area Konch 22 miles north west of Gaya Known samples 31 stray 11 (2) Area about 260 square miles type area Khizrsarai on the Phalgu 16 miles north east of Gaya Known samples 46 stray 36 (3) Area about 4 square miles type area bed of the river Morhar at Tekari 16 miles north west of Gaya Stray samples 22

Third tract of fertility —Area about 465 square miles type area Nawadah on the Khuri about 40 miles east of Gaya Known samples 55 stray 19

Southern Division Second tract of fertility —Area about 342 square miles type area investigated Nabinagar on the Punpun about 60 miles south south west of Gaya near where the Sone touches the district Known samples 35 stray 20

Third tract of fertility —Area about 287 square miles type area Rajauli on the Dhanarji 60 miles south east of Gaya Known samples 41 stray 17

Fourth tract of fertility —(1) Area about 160 square miles type area Sherghati on the Morhar about 22 miles south of Gaya and 800 feet over the sea level Known samples 37 stray 30 (2) Area about 300 square miles type area Imamganj an upland and forest area on the Morhar over 1 000 feet above the sea level and about 40 miles south west of Gaya Known samples 48 stray 21

Technique

The procedure adopted for making a stool suspension and counting the ova was that of Cort Grant and Stoll (1926) Briefly described the technique consists of weighing 5 grammes of fæces into a large sized test tube graduated at 75 c cs Sodium hydroxide in a decinormal solution, is poured in up to 75 c c mark and the tube stoppered The mixture is then vigorously shaken with glass beads until a homogeneous suspension has been obtained It is advisable to set up the tubes in the late afternoon for examination next morning A material in the quantity of 0 15 c c is then sampled with a graduated pipette and examined on a glass slide covered with a No 2 cover slip The total number of eggs found multiplied by 100 represents the number of eggs per gramme of fæces used The consistency of the stool can be placed in one of the three categories The mushy stool will readily stir with a spatula as compared with the formed stool while a diarrhœic stool will have to be handled with a spoon When using the method to estimate the degree of hookworm infestation the counts are all reduced to the basis of formed stool by multiplying the counts made on mushy stools by 2 and those made on diarrhœic specimens by 4

III Results Obtained in Field Studies

Hookworm Conditions

Northern Division The areas investigated were Jehanabad Daudnagar Konch Khizrsarai and Nawadah The results are shown in the known groups of population and in the order of the areas noted

Total positives 28 46, 28, 35 and 63, incidence 100, 81, 90, 75 and 97 per cent total mean degree of infestation 1,470, 1,230, 1,114, 857 and 1,778 ova per gramme

Landholding class cases, 66, positives 57, mean degree, 0, 1,258, 890 720 and 1,000 ova per gramme

Cultivating class cases 67, positives, 58, mean degree, 757, 1,350, 880, 1 050 and 3 033 ova

Landless labour class cases, 55 positives, 50, mean degree, 1,915, 1,325 1 320 600 and 1 607 ova

Artisan class cases 29, positives, 25, mean degree, 1,362, 771, 1,570, 200 and 1 366 ova

School going age cases 63, positives, 54, mean degree, 1,400, 1,259, 700, 667 and 850 ova

Wage earning age cases 147, positives 130, mean degree, 1,417, 1,210 1 191 897 and 2 331 ova

Stray fæces cases, 104 positives 71, incidence, 68 per cent mean infestation 937 ova per gramme Stray fæces at Tekari cases, 22, positives 6, mean degree 283 ova

Southern Division The areas investigated were Nabinagar, Rajauli, Sherghati and Imamganj The results are shown in the known groups of population and in the order of the areas noted

Total positives 27, 26 31 and 35, incidence, 77, 63, 84 and 73 per cent, mean degree of infestation 730, 719, 810 and 731 ova per gramme

Landholding class, cases, 33 positives, 23, mean degree, 762 0 650 and 700 ova

Cultivating class cases, 24, positives, 21, mean degree, 0 0, 760 and 900 ova

Labour class cases, 36, positives, 30, mean degree, 1,080, 1 044, 1,143 and 389 ova

Artisan class cases, 68, positives, 45, mean degree, 350, 517, 723 and 777 ova

School going age cases, 41, positives 27, mean degree, 714, 500, 520 and 554 ova

Wage earning age cases, 110, positives 86, mean degree, 782, 795, 865 and 843 ova

Stray fæces cases, 88, positives, 63, incidence, 72 per cent, mean degree 779 ova per gramme

The predominant species in the Gaya district area was *A duodenale* (*vide* Korke, 1926)

IV Discussion of Results

The subject matter is discussed under three headings —

(*A*) *Agricultural conditions in the areas investigated and the relation to hookworm infestation* —Broadly speaking the physical characters of Gaya are such as would tend to make the areas barren for the purposes of agriculture unless the soil is watered The degree of hookworm infestation has been expressed in terms of mean ova per gramme of fæces Cort and others (1926) have shown that the incidence statistics alone are of very limited value in expressing the hookworm situation for any area if the degree of infestation is not taken into account The areas under investigation were widely apart Owing to local conditions four areas in the northern division although belonging to the different tracts of fertility showed the highest degree of hookworm infestation which was more or less identical Similarly Khizrsarai and Sherghati Rajauli Imamganj and Nabinagar in the southern division showed the lowest degree of mean hookworm infestation which was more or less identical in individual cases The physical aspect of the ground and the degree of moisture retained in it are responsible for such results This is corroborated by the experimental evidence

In my study on the life history of hookworm (*N americanus*) in nature as affected by physical conditions of ground (Korke 1925 and 1926) I have shown the types of plots in which when once the ground was polluted by infected fæces infective stages of hookworm would or would not flourish The plot which showed abortive results had the physical characters of a sloped bed and the result of the experiment was that hookworm larvæ were present in fæces only and only rhabditiform stages were observed Superficial soil was negative for 12 days

The plot which showed infective stages well marked had the physical characters of ground commonly used for cultivation The result of this observation was that on the 5th day a rich brood of larvæ with well nourished mature stages were observed Between these two extremes four types of plots were described which showed larvæ in different morphological stages infesting the ground

Nearly one third area of the district has a markedly sloped surface and the rest is a comparatively level plain retaining considerable amounts of moisture These areas could favourably be compared with plots under experimentation Reading the results of the survey as a whole the mean hookworm infestation on the sloped surface was by 750 ova per gramme and on the level surface it was by 1 332 ova per gramme which figure is nearly twice in degree

(*B*) *Economic aspects and hookworm conditions* —The economic aspects incorporated observations on (*a*) the family and material conditions of the groups of agricultural class (*b*) the land value and sustenance and (*c*) groups of different ages

The evidence is in favour of two facts first that the groups of the agriculturists were infested according to their social grade and the frequency with which they came in contact with soil and secondly infestation was

proportional to the physical conditions prevailing in the northern and southern division

(*C*) *Fertility and hookworm conditions*—Grierson and Stevenson Moore have divided the land of Gaya district into four groups according to their varying degree of fertility and as fertility in this area is dependant mainly on the amount of water available and on the moisture with which the soil is bathed I have given hypothetical values for the degree of moisture in the different groups

Taking the first degree as 1, the second degree has been ascribed as 0 5 the third as 0 33 and the fourth 0 25 The mean fertile value per square mile for the five areas in the northern division is 0 69 and the total mean degree of infestation is by 1,332 ova per gramme Similarly the mean fertile value per square mile for the four areas in the southern division is 0 35 and the total mean degree of infestation is by 750 ova per gramme

The mean fertile value of the northern division is twice that of the southern division and the mean hookworm infestation in the northern division is also twice that of the southern division There appears to be a definite correlation between the fertility of an area as judged by the Grierson Moore standard of fertility and the hookworm condition

The direct relationship could also be checked in two ways —(*A*) By grouping the areas according to the physical agricultural and economic conditions, and (*B*) by taking an investigation along the course of the river Morhar whose indigenous canals feed the areas through the width of the district Nawadah Jehanabad Daudnagar and Konch would constitute one group, Khizrsarai and Sherghati as second group Rajauli Imamganj and Nabinagar as third group under (*A*) and Imamganj Sherghati Tekari and Jehanabad as one group under (*B*) The mean fertile value per square mile is 0 72 0 40 0 36 and 0 45 and the total mean ova per gramme are 1 400, 835 727 and 935 respectively The equational value in terms of one unit of fertility per square mile is approximately correct in each instance

Cort and others (1926) have correlated that 200 eggs per gramme per female worm or 100 eggs per gramme per hookworm gives a sufficiently accurate estimate of the probable worms harboured by a person

To translate the results in general terms it would be correct to surmise that in an area where the soil and moisture conditions are identical with the Gaya district area an average infestation of about 20 hookworms per person in the cultivating class of population would represent one unit of moisture or fertility of the soil of the first degree of the Grierson Moore classification

The evidence brought forward is therefore strongly in favour of the fact that not only is there a correlation between the degree of fertility which is entirely dependent on moisture conditions but that such a type of investigation tends to possess a diagnostic value The value of the results is highly serviceable to a practical sanitarian and is economical to the State

V Conclusions

1 There is correlation between the agricultural and economic conditions and the hookworm prevalence amongst the agricultural class in the Gaya district area The value of the correlation assumes a diagnostic significance

2 Moisture plays an important role in determining the hookworm conditions of an area A mean degree of infection by 20 hookworms per person (or 2 000 ova per gramme of fæces) would represent one unit of moisture or fertility of the soil of the first degree (Grierson Moore classification)

3 The mean degree of hookworm infestation amongst persons residing on a sloped surface was found to be less by half than that of a level surface

4 Under circumstances where establishment of a proper conservancy system is impracticable and where promiscuous defæcation unaided by conservancy has to be accepted as for the present inevitable the agricultural group of population may be encouraged to defæcate on a *dry sloped surface*

References

Cort and others (19 6) — *Amer Jour Hyg* Monographic Series No "

Grierson (1926) — Bihar Peasant Life Govt Printing Patna

Idem (1919) — Bengal Dist Gazette Gaya p 1"0

Korke (19"5) — *Ind Jour Med Res* Vol XIII No "

Idem (19"6) — *Ibid* Vol XIII No 3

Idem (19 6) — *Ibid* Vol XIV No 2

O Malley (1919) — Bengal Dist Gazette Gaya

Stevenson Moore (1898) — Report on the material condition of small agriculturists and labourers in Gaya Bengal Sectt Press Calcutta

THE CORRELATION BETWEEN ANKYLOSTOME DISEASE AND HÆMOGLOBIN VALUE AS OBTAINED IN REGARD TO THE PHYSICAL FEATURES OF AN AGRICULTURAL AREA IN INDIA AND TO THE SOCIAL AND ECONOMIC STATUS OF ITS POPULATION

BY

VISHNU T KORKE, F R C P (Edin), D T M (L'pool),
Central Research Institute, Kasauli

I. Introduction

The observations recorded in this paper were made during the course of hook worm investigations in the Gaya district area

The pathology of ankylostome disease is well known It will suffice to mention here that the disease is a toxæmic condition brought on by (*a*) predisposing factor and (*b*) hookworm infestation The toxæmic condition of blood has been observed from (*a*) the standpoint of deviation of mean Hb value from a 'standard,' the value of which has been determined in the district area of Gaya , and (*b*) from changes in the blood, which characterize the anæmias The predisposing factor has been studied from the standpoints of physical features and economic conditions The economic conditions have been censored in the light of sanitation, age, classes of agriculturists and conditions of family and land value, as are prevalent in the Gaya district area

All the information regarding the physical features of the Gaya district area, the social and economic status of its population and the value of the tracts of different fertility after the Grierson Stevenson Moore classification has been given in the foregoing paper

II Material and Technique

Material for observation was collected from the following groups of population —

(*A*) *Police Group, Gaya* —Fifty men were selected for observation They came out of agricultural classes which were better in social grade, comparatively well off in the material conditions under sanitary discipline and physically fit owing to the nature of vocation All were submitted to the Hb test

(B) *Raj School Group, Tekari* —Tekari is a municipal area and the school is maintained on the modern lines by the Maharaja of Tekari Two hundred and twelve boys between the ages 7 and 20 years were selected for observation, out of whom 102 were submitted to the Hb test and blood examinations The boys were drafted from 70 villages, 50 per cent of whom came from the neighbouring villages They mostly belonged to the landholding classes of agriculturists Broadly speaking, the defæcation in the case of boys who attended from villages was promiscuous especially outside the school hours

(C) *The agricultural sections of population* of Konch, Khizrsarai Nawadah and Imamganj areas, situated in the second third and fourth tracts of fertility in the terms of the Grierson Stevenson Moore classification Observations were made on 31, 46, 55 and 48 persons from the above areas respectively, out of whom 24, 33, 24 and 13 were submitted to the Hb test and blood examinations The groups of population were promiscuous in defæcation and free from sanitary control

The technique to determine the incidence and degree of hookworm infestation was that of Cort, Grant and Stoll (1926), and has been given in the previous paper The Hb tests were made by Gower's method and changes in the blood were observed by making the differential leucocytic counts and by noticing abnormalities in the red blood corpuscles The spleen census of the population submitted to the blood tests was made almost in all cases

III Results Obtained in Field Studies

Police Group, Gaya

The mean value of hæmoglobin for the adult age amongst the Hindus ranged between 70 and 73 per cent, amongst the Mohammedans 75 and 79 per cent, and for the total group 72 to 75 per cent in the negative and positive cases respectively, where the mean ages and weights were found to be identical

The mean degree of hookworm infestation amongst the Hindus was 616 ova per gramme, amongst the Mohammedans 130 ova per gramme, and for the total group 580 ova per gramme of fæces

No clinical signs and symptoms of hookworm disease were observed in the men belonging to this group The 'standard' mean hæmoglobin value for the adult age, 580 mean ova per gramme, showed no deterioration of the mean Hb value from the normal of 100 per cent as compared with the mean Hb value in the negative cases. The mean Hb value of 72 and 75 per cent, therefore has been accepted as the 'standard' for the negative and positive cases for the adult population of the agricultural group of the Gaya district area

Raj School Group, Tekari

The mean value of hæmoglobin for the juvenile age amongst the Hindus ranged between 68 and 70 per cent, amongst the Mohammedans 68 and 0 per cent, and for the total group between 69 and 79 per cent in the negative and positive cases

respectively where the mean ages were found to be identical The mean degree of hookworm infestation was 466 ova per gramme

No clinical signs and symptoms of hookworm disease were observed in the boys belonging to this group As in the case of adult age the mean Hb value of 69 and 70 per cent has been accepted as the standard for the negative and positive cases of the juvenile population of the agricultural group of the Gaya district area

AGRICULTURAL GROUPS FROM DIFFERENT AREAS

Khursarai Area —The mean degree of hookworm infestation was 881 and 760 ova per gramme in the adult and juvenile cases respectively The mean Hb percentage was 65 and 59 in the negative cases of adult and juvenile ages and 61 and 56 in the positive cases of the adult and juvenile ages respectively

The mean Hb percentage and ova per gramme in the landholding classes was 56 and 720 in cultivating class 65 and 1,038, in landless labourers 58 and 600 respectively No clinical signs and symptoms of hookworm disease were noticed in the cases of this group

Nawadah Area —The mean degree of hookworm infestation was 1 433 and 464 ova per gramme in the adult and juvenile cases respectively The mean Hb percentage was 54 and 56 in the positive cases of the adult and juvenile ages respectively The mean Hb percentage and ova per gramme in the landlord class were 59 and 1,010 in the cultivating class 50 and 600, in landless labourers 53 and 983 and in artisans 54 and 1 520 respectively No clinical signs and symptoms of hookworm disease were noticed in the cases of this group

Konch Area —The mean degree of hookworm infestation was 1,135 and 550 *ova per gramme in the adult and juvenile cases respectively* The mean hæmoglobin percentage was 47 and 49 in the positive cases of adult and juvenile ages respectively The mean Hb percentage and ova per gramme in the landholding class were 53 and 978, in the cultivating class 45 and 950, in labourers 43 and 1,267, and in artisans 50 and 450 respectively

Three cases of hookworm disease were noticed in this area belonging to landless labour class, two in adults and one in juvenile (*see* following Table) The blood condition and clinical picture were that of secondary anæmia

Imamganj Area —The mean degree of hookworm infestation was 1 287 and 380 ova per gramme in the adult and juvenile cases respectively The mean Hb percentage was 53 and 48 in the negative cases of adult and juvenile ages and 48 and 43 in the positive cases of the adult and juvenile ages respectively The mean Hb *percentage and ova per gramme in the landlord class* were 80 and 700, in the cultivating class 38 and 1,600, in the landless labour class 52 and 133, and in the artisan class 44 and 775 respectively

All the cases belonging to the agriculturist, landless labour and artisan classes showed clinical signs and symptoms of hookworm disease in the above area The blood picture was that of secondary anæmia The details of the cases are given in the Table

TABLE

Showing Cases in which Compensation for Blood Losses are disturbed and broken

Area	Hb per cent	Ova per gramme	Data ankylostome disease class and age		Differential Leucocyte Count P	I	L	I	H	Remarks
ıl	10	3 800	Landless L	°8 years						
mganj	10	4 800	Agriculturist	°5	54	14	3		9	I B C vacuolated No blood picture of primary anæmia in all the cases noticed
h	5	800	Landless L	5						
	35	300	Landless L	10	58	6			5	I B C [illegible]uolated
n_anj	40	80	Agriculturist	45	54	14			5	
	40	700	Agriculturist	10						
	45		Landless L	15	51	16	3		10	I B C vacuolated
	45	400	Landless L	6	54	1	4		10	
	45	? 000	Artisan	50	64	11	3		?	
	45	100	Landless L	14	7		8		8	
	50		Landless L	)	8	9	?		11	
	50	1 100	Agriculturist	)	6	1?	?	1	4	
	50		Artisan	3		[illegible]	?4	1	8	
	50	4?0	Artisan	1?		1?	1	1	7	
	50	300	Agriculturist	44	8	13	1	2	6	
	5		Artisan	55	54	8	?8	?	8	
	55		Landless L	35	8	1	?1		9	
	5-	100	Landless L	45	67		1	?	7	
	5?	?00	Landless L	4?	5	10	?3		9	
Total mean	44	848			58	1?	3	1	8	

IV Discussion of Results

The subject-matter has been discussed as follows —

A Hæmoglobin and Hookworm Conditions

Hookworm cases have been classified by Darling into three groups based on the degree to which compensation for blood losses occurs Group A blood loss compensated Group B compensation disturbed and breaking, Group C compensation broken

In the China Hookworm Survey, Cort and others (1926) have found that negatives to dilution egg count (a natural group) to positives showing a mean count of 200 ova per gramme yielded a mean Hb value 67 to 70 per cent, and positives with a mean count of 1 700 ova yielded a mean Hb value 62 4 per cent The above series fell within the limit of Darling's Group A Positives with a mean count of 6 500 ova and mean Hb 55 per cent also all positives showing 10 000 ova per gramme and mean Hb 50 to 40 per cent fell within the limit of Darling's Groups B and C

Both the China and Gaya investigations agree with the results in regard to the correlation between the Darling's three groups and the mean Hb value For instance 70 and 68 is the mean Hb in the positive and negative cases where the positives showed a small sized count of mean ova, 60 to 55 is the limit of mean Hb in Group A 55 to 48 in the Group B and 46 to 40 6 in the Group C But the difference in the result lies in the fact that the range of variation of mean ova in the *China series is very great in order to explain the correlation between the value of* Hb and the different groups of Darling

The conditions of Gaya differed materially from China As a rule no human fertilizers were used by the agriculturists as perhaps was done in China and which condition accounted for a larger mean count of ova per gramme In Gaya the total mean degree of ova per gramme never exceeded 1 500 and yet the blood conditions as is expressed in the three groups has been reached Moreover no direct relation was observed between the number of ova and the fall in the Hb value

The explanation for such a result has to be sought for in a predisposing factor which undoubtedly is the economic condition of the agricultural classes residing in the different tracts of fertility On this basis a correlation between the Hb value and hookworm infestation is obtained

The standard Hb value which has been obtained in the cases of adult and juvenile ages has been the outcome of economic conditions of health and disease other than the condition of hookworm The value thus obtained is 70 and 75 per cent in the positive cases and 69 and 72 per cent in the negative cases of juvenile and adult ages respectively

Judging from this standard, Khizrsarai showed the deviation of 14 and 14 points Nawadah 14 and 21 points, Konch 21 and 28 points and Imamganj 27 and 27 points in the positive cases of juvenile and adult ages respectively No

clinical cases of ankylostome disease were found in Khizrsarai and Nawadah; three cases were found in Konch and 16 out of 17 cases, showing well marked signs and symptoms of the disease, were found in the Imamganj area There was no direct correlation between the mean degree of hookworm infestation and the fall of hæmoglobin value from the standard But the fall of Hb value was proportional to the economic conditions of the agricultural classes in the above areas

B The Economic Conditions of an Area and the Hookworm Disease

The areas of Khizrsarai, Nawadah, Konch, and Imamganj stood in due order of merit In the first area 15 belonged to the landholding and 16 to the pure agriculturist class, result no cases of hookworm disease In the second, 19 landholding and 5 cultivating class, result no cases In the third, 9 landholding, 4 cultivating and 11 labour and artisan classes result 3 cases in the labour class The fourth area, situated in the infertile tract, presented all cases of hookworm disease It is obvious, therefore, that the economic conditions of an area are in direct relation to the predisposing factor which precipitates the onset of ankylostome disease by comparatively a minimal sized count e g 1 038 to 938 mean ova per gramme in the different groups of agricultural population

C The Classes of Agricultural Group and Hookworm Disease

The deviation of mean Hb value in points from the standard value runs as the following series Landholding, 16 points, agriculturists, 17 points, landless labourers 24 points, and village artisans 25 points There were 5 cases in agriculturists, 7 cases in labourers, 4 cases in artisans, which showed clinical manifestations of hookworm disease, and out of the total cases, 11 were contributed by one area alone which was very poor in the economic conditions

To translate the results in general terms, therefore it is reasonable to presume that, where the deviation of hæmoglobin from the standard value in the district of Gaya is between 14 to 21 points, the groups of population fall within Darling's Group A, both in the cases of adult and juvenile ages Similarly where the deviation is between 21 to 28 points from the standard, the groups of agricultural population fall within Darling's Group C Between these deviations from the standard value should lie the value of Darling's Group B

The type of the investigation possesses a practical value for a sanitarian and to the State

V CONCLUSIONS

(1) That the predisposing factor of the ankylostome disease is in direct relation to the physical features of an agricultural area and to the social and economic status of its population, which conditions express the degree of resistance of the groups of population for the ankylostome disease

(2) That the group resistance is expressed in the terms of mean Hb value which is in direct relation to the economic condition prevailing in an agricultural area

(3) That a comparatively small degree of mean hookworm infestation was sufficient to bring on the onset of ankylostome disease in a group of population whose resistance has been lowered by the economic conditions

(4) That in the groups of agricultural population the landless labour class was first to succumb to ankylostome disease especially in an area whose economic condition surrendered a poor value

(5) There was no direct correlation between the mean degree of hookworm infestation and the ankylostome disease in the agricultural groups of population except through the medium of the agricultural features and the social and economic status of its population

REFERENCES

CORT and OTHERS (1926) *Amer Jour Hyg* Monographic Series No 7

GRIERSON (1926) Bihar Peasant Life' Govt Printing Patna

O MALLEY (1919) Bengal Dist Gazette Gaya

STEVENSON MOORE (1898) 'Report on the material condition of small agriculturists and labourers in Gaya Bengal Sectt Press Calcutta

PRELIMINARY OBSERVATIONS ON ANKYLOSTOMIASIS IN PARIAH DOGS

BY

GOVIND SINGH THAPAR, M SC, PH D (Lond)
Offg Professor of Zoology, The University, Lucknow

In the course of my investigation on the helminth parasites of pariah dogs in Lucknow, I came across a few nematode worms from their intestines, along with a large number of cestodes These worms on examination, turned out to be *Ankylostoma duodenale* Although its presence has been recorded from 'Felidæ,' so far as I am aware, this parasite has not been reported from pariah dogs in India This led me to a further examination of a number of dogs and all of them were found infected with the same parasite, the host however, showing the usual symptoms of weakness, etc It further indicates that, in the spread of hookworm disease in man, the agency of the pariah dog—an animal so commonly associated with us in our homes—cannot be long ignored

DISCUSSION

Mr J T Edwards (United Provinces) We have carried out a number of investigations at Muktesar during recent years with the object of finding an effective method of treatment for hookworm disease in dogs We had reason to believe that hookworm infection was common and widespread in this species in India and in fact, I can recall the case of a pariah puppy at Muktesar that was suffering from a rapidly progressive anæmia in which the egg count amounted to about 400 per gramme of fæces The mature worms were also commonly found often in considerable numbers in post-mortem examinations on dogs For our investigations on treatment, numbers of pariah dogs were obtained from the Bareilly district I am unable to express an opinion on the helminthological identity of the worms but my zoological assistant, Mr Amar Nath Gulati, who devoted some attention to this matter assured me that the species resembled more closely *Ankylostomum ceylanicum* than *A caninum* In any case, no report of the discovery of *A duodenale* was made to me

The work was of further interest in that our results in experimental treatment did not coincide with those of Hall and his associates in America Our technique differed somewhat from that adopted by these authors, who, after administering treatment, sacrificed their dogs and ascertained the so called anthelmintic efficacy of the treatment by comparing the number of residual mature worms in the intestine with those originally

present, calculated by adding the numbers of worms expelled to those remaining We made egg counts of the fæces for several days before treatment, which were then continued for a sufficiently long time afterwards, before using the same animals for a further experimental treatment The curves so obtained showed that carbon tetrachloride, oil of chenopodium, alone and in combination, given with castor oil or magnesium sulphate, according to the indications given by Hall exercised no permanent effect upon the infection For some days, the egg count was markedly lowered, but it invariably became restored later, apparently indicating temporary injury to the mature emales which would retard their egg laying capacities The difference in our observations from those recorded by the American workers is difficult to interpret It may be attributed to the fact that the infection was generally a slight one or to some specific differences in the parasites themselves, which, in any event, need further elucidation

Major H Stott, I M S (United Provinces) Mr Edwards' important observations on the experience of his research department at Muktesar and Dr Thapar s report on hookworms seem to me to be most important, for if infection of pariah dogs in India is proved to be common, these animals will have to be seriously considered as carriers of infection

Such an experience has not come my way I can only speak of one case in which in a pedigree dog and in a pariah dog, hookworm ova were found in my Department of Pathology at Lucknow What variety of hookworm this was I cannot say The eggs disappeared on treatment

Dr P A Maplestone (Bengal) (1) Does Dr Kendrick know the condition of prisoners on arrival in gaol, because they are probably under better conditions in gaol than in their own villages and this may lower the actual effect of Ankylostome infection as observed by him ?

(2) As the artificial infections were done in one day and were massive, may not the natural resistance expected to be acquired by slow natural infection be broken down more completely by these massive infections ?

With reference to Dr Thapar's paper before accepting this worm as *A duodenale* cross infection experiments would have to be done from dog to man or vice versa because morphology is not the only criterion of diagnosis in helminths from animals of different groups This is seen in the failure to infect pigs with *N americanus* although a worm, morphologically identical, has been found in Trinidad in pigs by Ackert and Payne and also by Gordon in the Amazons and Jamaica

The failure to perform successful cross infections between the ascaris of the pig and man, which are identical in appearance, have been very numerous This shows that there are two distinct species in races which cannot be separated by microscopical examinations

Dr V G Heiser (U S A) In view of the relationship which has been shown to exist between rainfall, sub soil water and stool habits one wonders whether the deductions drawn by Dr Korke for the Gaya district would be applicable to other agricultural areas, without considering the foregoing factors

Dr H Kobayashi (Japan) I am very glad to have the honour to hear the researches on parasites in India Further, I am very deeply interested in Dr Sweet s

and Dr Korke's work, especially that of Dr Korke, who has been considering the parasitic diseases from the geo morphological point of view I am studying the same subject in Japan, fluke diseases, hookworm disease, malaria and others both in Japan proper and in Korea The parasites live for a certain period, in the free state or in the body of certain animals, intermediate hosts These stages or these animals are under the influence of the geographical features I expect in future, that this problem will occupy a very important part in the study of parasitology

Dr T S Tirumurti (Madras) Dr Korke said that a knowledge of the relation of *Ankylostoma* to physical features is of diagnostic value I wonder of what diagnostic value it is to the clinician The only way of diagnosing infection is to find the eggs in the motions or examine the motions for worms after anti hookworm treatment I request that the diagnostic value of his observations may be explained

Dr J F Kendrick (Madras) In reply to Dr Maplestone's question regarding the condition of the men who volunteered to be re infected, I have to say that the men were apparently in good health and were known to be hookworm free

It was suspected that a sudden infection with a considerable number of larvæ might be more serious than an infection that was slowly acquired but in any case a given number of Ankylostomes is always more injurious than an equal number of Necators

Dr V T Korke (B India) replied The fertility of an area denotes the factors of rainfall, sub soil water, etc Promiscuous defæcation in the agricultural classes of India denotes stool habits The paper is of diagnostic value as foretelling the hookworm condition of any area to a similarly situated area in the Gaya district

PARASITIC INFECTIONS IN THE FOOCHOW AREA, FUKIEN PROVINCE, CHINA *

BY

ERNEST CARROLL FAUST, M A, PH D

AND

CLAUDE R KELLOGG, M A

FOOCHOW, the capital and metropolis of Fukien Province, lies in the mouth of the Min Kiang, the tributaries of which drain practically all of the northern half of the province The city proper is situated on the north bank of the river, several miles inland from the sea, while a large island in the middle of the river faces the city from the south The Foochow plain is flat country, consisting for the most part of paddy, intersected at intervals by steep mountain ranges coming down to the sea In these mountains there is a luxuriant natural growth of bamboo, sword grass and tiger grass among which tigers, leopards wild pig and serow are not infrequently encountered Within visual distance to the south of Foochow are the mountain recesses from which the blue tigers (i e, maltese tigers) have been reported Further up the Min River at Yenping and still more so at Shaowu to the north west and Kienning to the north, the country consists of steep mountain ranges with swiftly flowing streams in the valleys the whole supporting a luxuriant growth of semi tropical vegetation The mean average temperature and the mean average rainfall are such as to justify the inclusion of this region in the moist sub tropical belt

During the summer of 1927 approximately 500 samples of human stools from several villages in the region were collected and examined Likewise, stools of possible reservoir hosts of human infection were also studied In addition, several dogs and cats and numerous snakes and frogs were autopsied and examined for parasites The survey was the more desirable in view of the fact that no similar investigation had been previously made in Northern Fukien Province

*Contribution No 88 from the Parasitology Laboratory Department of Pathology Peking Union Medical College

Determination of the Incidence of Parasitic Infection in the Human Population

This was based primarily on the examination of human fæcal specimens The villages from which the material was obtained may be roughly divided into four groups consisting of (1) those in the rice growing area of the Foochow plain between the city and Pagoda Anchorage (2) those in the mulberry district several miles above the city (3) a mountain district (Peiliang) to the north of Foochow where rice and tea are grown and (4) two Hak Ka villages in the mountains to the north beyond Peiliang The material was collected from freshly passed specimens placed in glass specimen bottles and taken to Foochow where it was carefully examined in the zoological laboratory of Fukien Christian University Three microscopic slide preparations of each specimen were studied a few drops of eosin iodide solution being run under one side of the cover glass to facilitate the detection and diagnosis of the protozoa In the case of certain pooled samples of fæces from night soil pits in the Hak Ka and Chinese mountain villages the hookworm eggs had already hatched apparently several days previous to the time of collection so that when the samples were examined filari form larvæ were already present This made it possible to determine with considerable accuracy the species of hookworm present using the technique described by Svensson and Kessel (1927) The following table (Table I) gives the important findings in the series

TABLE I

Data on the incidence of human parasitic infections in the vicinity of Foochow Fukien Province China determined by fæcal examinations

Species of parasite	Rice villages		Mulberry villages		Mountain village (Chinese)	Mountain villages (Hak Ka)	
A PROTOZOA	Incidence per cent		Incidence per cent		Incidence per cent	Incidence per cent	
	(A)	(B)	(A)	(B)		(A)	(B)
Endamoeba histolytica	7 6	1 0	3·0	1·0	3 3	3 6	3 3
Endamoeba coli	5·9	14·0	3·0	5·0	6 6	5 4	4 7
Councilmania lafleuri	0	4·0	2·0	4 0	3 3	1 8	2 3
Endolimax nana	3 8	8·0	6 0	7·0	4 [illegible]	3 6	2·3

TABLE I—*concld*

Species of parasite	Rice villages		Mulberry villages		Mountain village (Chinese)	Mountain villages (Hak Ka)	
A PROTOZOA	Incidence per cent		Incidence per cent		Incidence per cent	Incidence per cent.	
	(A)	(B)	(A)	(B)		(A)	(B)
Iodamœba butschlii	0	2 0	0	1 0	0	1 8	0
Giardia lamblia	1 9	0	0	3 0	2 17	1 8	0
B HELMINTHS							
Ascaris lumbricoides	93 1	76 0	63 0	54 0	93 3	81 8	100·0
Trichuris trichiura	19 0	36 0	14 0	12 0	4 35	11 7	19 2
Ancylostoma or Necator	0	4 0	12 0	2 0	8 70	56 9	55 2
Strongyloides	0	0	10 0	2 0	10 88	5 8	1°0
Schistosoma japonicum	0	0	0	0	2 17	0	2 4
Fasciolopsis buski	0	4 0	0	0	0	0	0
Enterobius vermicularis	0	0	0	0	0	1 5	0
C ARTHROPODA							
Acarinid eggs	0	0	0	1 0	0	*0*	*0*
Total protozoan incidence per cent	14 5	40 0	14 0	21 0	34 4	15 4	15 5
Total helminth incidence per cent	112 1	1°0 0	102 0	70 0	108 8	160 8	190·7
Total parasite incidence per cent	126 6	160 0	116 0	91 0	143 2	176 2	°06 °

Analysis of the data provides the following information as probably typical for the area —

(1) The relatively low incidence of intestinal protozoa including *Endamoeba histolytica* as compared with areas in Central and North China

(2) The consistently heavy infection with *Ascaris* and to a somewhat lesser extent of *Trichuris*, these infections representing direct contamination of the human population with the night soil

(3) The consistently low incidence of hookworm infection on the plain including the mulberry villages as well as in the Chinese mountain village and in contrast the high percentage of hookworm infection in both of the Hak Ka mountain villages

(4) The complete absence of tapeworm infection in the data obtained directly from the survey

(5) The incidental infection with *Schistosoma japonicum* in the mountains and with Fasciolopsis on the plain

(6) The complete absence of fluke infections incurred from consumption of raw fresh water fish

These data call for certain explanations in order that they may not be misinterpreted In the first place they are based on three examinations of a single stool specimen from each individual Experience in China has shown (Faust 1924 Kessel and Svensson 1924) that careful examination of a single stool specimen makes it possible to detect on the average about fifty per cent of the total infection (based on six consecutive stool examinations) with a particular species of parasite in the human population In order therefore to make suitable comparisons with data obtained from other areas in China the respective data from the several areas cited have been reduced to the six examination basis These comparable findings are set down in Table II These data indicate that the percentages of incidence of intestinal protozoa in the Foochow area are all lower than those in the Peking or Wuchang surveys they belong in the same series as the Canton data published here for the first time The percentage of incidence of intestinal helminths among the Chinese population in the Foochow district is considerably higher than that in the Peking population but is markedly lower than that in the Wuchang population On the other hand the average percentage *incidence of intestinal helminths in the Hak Ka villages* even though the number of species is few *represents the highest index thus far discovered for any area in China*

An explanation for certain of the data presented is to be found in the topographic and meteorological conditions prevailing in the Northern Fukien district in the habits of the human population and in local factors bearing on the life-cycles of the various infections These several points will be taken up under each group or species of parasite wherever they have a direct bearing on the findings

TABLE II.

Synoptic data on the incidence of intestinal protozoan and helminthic parasites occurring in man from representative areas in China, reduced to six examinations

Region	Parasite group	Total percentage incidence of infection	Authority
Peking	Protozoa	101 60	Faust, 1924
	Helminths	88 02	Faust, 1924
Wuchang	Protozoa	304 80	Faust and Wassell, 1921
	Helminths	312 40	Faust and Wassell, 1921
Swatow	Protozoa	incomplete data	.
	Helminths	198 00	Faust survey, 1925 (unpublished data)
Canton	Protozoa	38 60	Faust survey, 1925 (unpublished data)
	Helminths	138 40	Faust survey, 1925 (unpublished data)
Foochow rice villages	Protozoa	53 20	Present survey
	Helminths	234 00	Present survey
Foochow mulberry villages	Protozoa	35 00	Present survey
	Helminths	172 00	Present survey
Foochow mountain village (Chinese)	Protozoa	68 80	Present survey
	Helminths	217 60	Present survey
Foochow mountain villages (Hak Ka)	Protozoa	30 80	Present survey
	Helminths	344 20	Present survey

Protozoan infections—In only three of the nearly five hundred cases examined were the trophozoite stages of protozoa encountered (two instances of *Endamœba histolytica* and one of *Giardia lamblia*) No cases were found exhibiting infection with Chilomastix Trichomonas or Embadomonas or of the coprophagous forms Cercomonas and Bodo On the other hand the occasional presence of mononucleate and binucleate cysts of *Endamœba histolytica E coli* and Councilmania indicated that the stools were fresh Hence the low incidence of parasitic protozoa almost exclusively in the encysted stage deserves consideration The fact that a low incidence of these protozoa was encountered in all of the villages from which sample were obtained and that a similarly low index of infection was obtained by the writer in Canton (March 1925) in a mixed population suggests the possibility that chronic and carrier cases of these infections are fewer in South China than in Central or North China If this condition is counterbalanced by a higher number of acute protozoan infections in which the trophozoite stage was exclusively or predominantly present such a state must have prevailed at some other period than that when the South China surveys were made since in no cases in the Fukien series were the stools dysenteric in character while only three of those in the Canton series (of approximately five hundred) were characteristic of amœbic dysentery The small amount of promiscuous defæcation observed both in the Foochow region and in Canton city and the long ripening of the night soil in the former area may be contributing factors to the low incidence but the main underlying reason must be more fundamental and far reaching Further data of a more extensive nature must be accumulated before a satisfactory solution is provided for this significant finding

Ascaris and Trichuris infections—The consistently heavy infections of *Ascaris* and *Trichuris* in the cases examined was not unexpected The moisture and temperature conditions of the area are favourable for the extra human development of the embryos *in ovo* while the ripening process of the night soil does not materially reduce their viability

Hookworm infections—The low incidence of hookworm infection in the rice growing district near Foochow conforms to the general findings of the China Hookworm Commission (Cort Grant and Stoll 1926) that paddy *per se* is usually not a source of hookworm infection On the other hand the light infection in the mulberry area in the Foochow district is in striking contrast to the findings of the China Hookworm Commission for the Foochow and Canton districts Several possible reasons may account for this difference In the Foochow area mulberry is not raised as extensively or as exclusively as in the two districts studied by the China Hookworm Commission More important however is the fact that there is almost no direct contamination of the ground around the mulberry with freshly passed (i e unripened) human fæces Throughout the whole of Northern Fukien the night soil is very carefully collected as liquid compost poured into deep rectangular stone lined pits and ripened over a period of several months The clayey soil around the pits does not permit of seepage The ripening process is

sufficiently lengthy to destroy the hookworm larvæ so that the eggs when ladled out of the pit with the ripened manure are non viable This process of ripening is made possible by an abundance of night soil pits so that fresh night soil is not poured into pits containing the nearly ripened fertilizer The population has therefore developed a custom of night soil ripening which is highly efficient in rendering the hookworm eggs non viable, although the eggs of *Ascaris* and *Trichuris* are still viable when the manure is placed on the fields

While the incidence of hookworm infection in the Chinese villages in the vicinity of Foochow is uniformly much lower than might be expected when the environmental factors are considered the two Hak Ka villages from which samples of fæces were taken proved to be peculiarly adapted for the heavy infections found among their inhabitants In the first place it may be stated that these people are racially different from the Chinese population surrounding them They have more primitive customs speak a different dialect, and never mingle with the Chinese except to barter and trade They invariably live in mountainous regions Of the twenty one centres in Fukien Province in which these villages are found the two villages visited were nearest to Foochow (about 30 miles distant) The squalid thatched homes in which the villagers live were situated on terraces somewhat above the bottom of the narrow mountain valley The winding pathway from the main mountain trail to the settlement was difficult to negotiate particularly in wet weather when one sank ankle deep in the slippery mud The path first led down into the bottom of the valley and then up the side of the terrace At the head of the terrace before coming to the homes the pedestrian passed the village latrines some of which were situated on the upper side and some on the lower side of the path The path itself was nearly impassable due for the most part to seepage from the latrines on the upper side Since the natives are invariably barefooted or at most only shod with straw sandals and since they are obliged to use this path at least twice daily on their way to and from the fields which they cultivate an ideal means is afforded for human infection with the filariform hookworm larvæ which were found to be incubating in the muck As the data in Table I indicate the incidence of hookworm in these villagers is high (56 9 and 55 2 per cent respectively) examination of pooled samples from several of these latrines showed that both hookworm and Strongyloides larvæ were very abundant particularly along the *edges of the latrines near the overflow into the muck of the village pathway* Thus there is good epidemiological evidence pointing to a vicious cycle of hookworm and Strongyloides infection in the villages studied

Opportunity did not permit for making cultures of the individual samples obtained in order to determine whether the larvæ developing from the hookworm eggs were those of Ancylostoma or Necator However in the large samples taken [illegible]

shown to be diagnostic for these respective species a study was made o[illegible] hundred of these larvæ first differentiating them from the free living and the

Strongyloides species and then studying their more specific characters *In every single specimen studied the filariform hookworm larvæ proved to be those of Ancylostoma* The absence of dogs from the immediate vicinity of the villages precludes the possibility that the larvæ were those of *A caninum* The fact that the tropical hookworm *A braziliense* has never been encountered in China or Formosa in man dogs or cats argues against the larvæ belonging to this species Evidence therefore favours the view that the Ancylostoma larvæ found were those of *A duodenale* The information is the more valuable in view of the fact that the predominant hookworm infection among the Southern Chinese including those from Foochow is Necator While *Ancylostoma duodenale* is almost exclusively the hookworm found in Japan and is probably the more common species in North China it is much less frequently encountered in Central and South China than is Necator The explanation offered for the apparently pure culture of *Ancylostoma duodenale* in isolated foci in a region where Necator is predominantly the human hookworm species involves a consideration of the isolation of the Hak Ka villagers from the Chinese people who surround them on all sides The condition is not unique Soper (1927) has recently found a nearly pure *Ancylostoma duodenale* infection among native Paraguayan Indians who have remained in isolation from the outside world The inference in both cases is that the Ancylostoma infection is autochonous while Necator is an introduced species

Tapeworm infection —In no case of the series examined was tapeworm infection encountered The thorough cooking of pork practically precludes *Tænia solium* *T saginata* if present in the Fukienese population is very rare Cases of *Hymenolepis nana* have not been reported from the Province No autochonous case of echinococcus infection has been positively diagnosed from Central or South China The adult stage of Manson's tapeworm (*Diphyllobothrium mansoni*) occurs in man in Central China but has not been found in Fukien Province although the larval stage *Sparganum mansoni* was first described from Amoy The abundance of this larval type as a somatic infection in frogs and snakes and the presence of the adult worm in cats in the Foochow area together with the experimental proof of the genetic relationship of larvæ and adult worms have been established by us during this survey Furthermore Dr Horace E Campbell of Pagoda Anchorage, soon after learning of our interest in Manson's tapeworm and of its possible presence in man extracted a specimen of the Sparganum stage from an ulcerated thumb of a native of the vicinity In view of the fact that frogs' legs and snake flesh, incompletely broiled or fricasseed is a great delicacy among the Foochowese, infection with the adult *Diphyllobothrium mansoni* is not unlikely

Schistosoma infection —The data indicate the presence of *Schistosoma japonicum* in the Peiliang region Furthermore examination of fæces of water buffaloes on the Foochow plain has provided us with one positive finding in this host, the first record of the infection in this animal The disease has been previously reported from the vicinity of Pagoda Anchorage (report of Dr Chas Gilette in Faust and

Meleney 1921) It is also known to be prevalent at Hinghwa on the Fukien coast some distance to the south of Foochow

Fasciolopsis buski infection —A light infection with this fluke has been found by us in the villages adjacent to Foochow city A number of cases have also been recently diagnosed from within the city The water chestnut (*Eliocharis tuberosa*) appears to be the source of the infection The low incidence is possible due to peeling off the skin of the corn with a knife rather than with the teeth before eating the vegetable raw Pigs in Fukien Province are commonly infected with this fluke

Infection with flukes introduced through the consumption of fresh water fish There is no record of autochonous cases of human infection with *Clonorchis*, *Metagonimus* or *Echinochamusmus* in Fukien Province Cats in Foochow were found lightly infected with Clonorchis but dogs were negative

Infections in Reservoir and Intermediate Hosts

The intestinal protozoan infections of the various animals which might serve as the reservoir or intermediate hosts of human infections were in no case found to be closely related to the human intestinal protozoa

Among the cats autopsied 45 per cent harboured *Dipylidium caninum* 36 per cent were infected with the adult *Diphyllobothrium* and 27 per cent were positive for *Clonorchis sinensis* The dogs autopsied were found to be infected with the following potential human helminths *Dipylidium caninum* 46 per cent *Diphyllobothrium* 9 per cent Twenty six per cent of the dogs also harboured the human flea *Pulex irritans* Native goats had a 12 per cent infection with *Trichostrongylus* and an equal amount of *Fasciola hepatica* The native cow was found to be constantly infected with *Fasciola hepatica* The water buffalo harboured a 37 per cent infection with *Fasciola gigantica* while one case of infection with *Schistosoma japonicum* was found Many of these helminthic infections have been previously encountered in domestic animals in South Fukien Province (Maxwell 1921)

From the autopsy of possible intermediate hosts of human helminth infections the following records were obtained In *Rana tigrina* Manson's Sparganum was obtained as an infection of the somatic musculature in 63 per cent of the cases *R nigromaculata* was negative *Microhyla sowerbyi* had a 9 per cent infection with the larva *Bufo melanostictus* was consistently negative *Natrix tigrina* always carried a very heavy infection with this helminth as did *N piscator* On the other hand the 'yellow snake' and the 'red bellied snake' were never found infected with Sparganum Among the potential crustacean hosts of Paragonimus infection several dozen specimens of *Potamon delaani* were examined but were all found uninfected Pulmonary distomiasis has not been definitely proved for Fukien Province although it is stated on good authority that autochonous human cases occur in the vicinity of Kienning above Foochow Certainly tigers are common enough in the Min River valley to serve as a common reservoir of the fluke

Conclusions and Summary

The survey to determine the amount of infection with human intestinal parasites in the Foochow area shows that the number of protozoan and helminthic infections is surprisingly small when compared with similar data from Central and North China. The number of species of animal parasites infecting the human population is extremely few in contrast to the number known to be present in Central China (Faust and Wassell 1921) and in the lower Yangtze drainage. Furthermore the incidence of infection particularly as concerns the intestinal protozoa is also very low in Northern Fukien. The only exceptions to these general statements are *Ascaris* and *Trichuris* infestations which are uniformly high and hookworm disease in the Hak Ka villages. The reasons for this low percentage of infection in the Foochow population with species of the parasites so frequently encountered in the Yangtze Valley are not clear. This is particularly true with respect to the intestinal protozoa. Possibly the topography of the country, limiting the spread of the parasites and their intermediate hosts may contribute to prevailing conditions. Undoubtedly the customs of the human population with respect to the manner and methods for ripening night soil is responsible for the low incidence of hookworm infection. Likewise, abstinence from eating raw pork beef fresh water fish and crustaceans explains the low amount of cestode and trematode infections. Nevertheless the low incidence of Clonorchis in cats and its absence in dogs as contrasted with the overwhelming infestation of this fluke in these reservoir hosts in the Yangtze drainage indicates that the infection is not even a serious potential human infection in Northern Fukien. The comparatively low incidence of most of these infections in Swatow and Canton to the south of Fukien Province provides further grounds for believing that conditions in coastal South Eastern China are on the whole less favourable for the development of the large number of species and high incidence of infection with human protozoa and helminths than they are in the Yangtze Valley. Of these factors it seems most reasonable to conclude that topographic and meteorologic conditions are the most significant although the exact way in which they operate will require additional study.

The following important facts have been obtained from the survey of human parasitic infections in the Foochow area —

(1) The small number of species and low incidence of intestinal protozoa.

(2) The uniformly heavy infection with *Ascaris* and to a somewhat lesser degree of *Trichuris*.

(3) The low (sub clinical) incidence of hookworm infection in the area except in the Hak Ka villages near Foochow where a heavy infection of apparently pure *Ancylostoma duodenale* was encountered.

(4) The absence of Taenia Echinococcus Hymenolepis and Dipylidium infection in the human population, the occasional presence of *Sparganum mansoni* in man and the possibility of human infection with the adult *Diphyllobothrium* due to the high infectivity of the

intermediate hosts commonly consumed as food without sufficient heating

(5) The incidental infection with *Schistosoma japonicum* and *Fasciolopsis buski* in the vicinity of Foochow

(6) The complete absence of Clonorchis infection in man and in dogs and its low percentage of infectivity in cats

On the whole, it seems reasonable to conclude that human intestinal parasitic infections are not clinically as significant in Northern Fukien as they are in the Yangtze Valley Individuals may not infrequently manifest clinical symptoms due to infection with a particular species of parasite and under certain local conditions a whole community may be seriously affected by a particular species but the area as a whole is much less heavily parasitized than had been previously supposed The significance of these findings cannot be over estimated in considering the noso geography of human intestinal infections in China

REFERENCES

CORT, W W, GRANT, J B and STOLL, N R (1926) Researches on hookworm in China *Amer Jour Hyg* Monogr Ser No 7 p 398

FAUST, E C (1924) Observations on North China intestinal parasites of man *Amer Jour Trop Med*, Vol IV, pp 411—437

Idem with MELENEY H E (1924) Studies on Schistosomiasis japonica *Amer Jour Hyg* Monogr Ser, No 3 pp 336 pl 36

Idem with WASSELL, C M (1921) Preliminary survey of the intestinal parasites of man in the Central Yangtze Valley *China Med Jour* 35, pp 532—561

KESSEL J F and SVENSSON, R (1924) A survey of the human intestinal protozoa in Peking China *Ibid*, 38, pp 961—982 tab 8

MAXWELL, J L (1921) Intestinal parasitism in South Fukien *Ibid* 35 pp 377—382

SOPER, F L (1927) The report of a nearly pure *Ancylostoma duodenale* infestation in native South American Indians and a discussion of its ethnological significance *Jour Parasitology* 13 p 226

SVENSSON, R and KESSEL, J F (1926) Morphological differences between Necator and Ancylostoma larvæ *Ibid*, pp 146—153 pl I

FILARIA MALAYI N. SP. PARASITIC IN MAN IN THE MALAY ARCHIPELAGO

BY

S. L. BRUG

Geneeskundig Laboratorium, Weltevreden

I. INTRODUCTION

UNTIL recently the common opinion was that in the Malay Archipelago only one species of human filaria occurred, viz. *Filaria bancrofti*. Wherever microfilariæ were observed in man they showed a nocturnal periodicity and a large sheath. Where a great part of the population was found infected with filaria, elephantiasis occurred more or less frequently. So the common opinion that every filaria observed in this country was *Filaria bancrofti* seemed to be well founded.

Now in 1926 Lichtenstein (1927) studied filariasis in Bireuen (Atchin, Sumatra) especially with a view to determine the carrier. He infected some 300 mosquitoes, most of which were *Culex fatigans*, with the blood of persons harbouring microfilaria. He was very surprised to find hardly any development of the ingested worms in the mosquitoes, or even none at all. Sometimes a larva could be traced amidst the thoracic muscles, but the typical half grown sausage shaped stage was never seen.

Moreover it struck this author that, although filarial infection was far from rare in Bireuen and its vicinity (filarial index some 30 per cent to 50 per cent), only elephantiasis but no other pathological lesion of filariasis was ever seen. A special research of a great number of inhabitants from badly infected villages failed to reveal any case of filarial disease other than elephantiasis. Dr. Verhagen, working also in Atchin and well acquainted with filarial disease by a long stay in Surinam, made the same observation.

Apparently the Bireuen filaria differed in two respects from *Filaria bancrofti*: (1) it did not develop in *Culex fatigans*, and (2) it was unable to bring about any filarial symptom other than elephantiasis. So it seemed very doubtful whether the Bireuen filaria really was *F. bancrofti*.

Adult worms not being obtainable, Dr. Lichtenstein kindly sent me some thick film slides containing microfilaria from autochthonous Bireuen cases. I shall not discuss separately the results of the examination of these slides, but shall consider them in connection with what I observed in slides from other parts of the Malay Archipelago.

I am much indebted to Drs van Beek, Soesilo Lichtenstein Sardjito Karanos and Erber for thick films from Pantar (Lesser Sunda Islands) Nias (Island west of Sumatra), Malang (Java) Palembang (Sumatra) Makassar and Mamoedjoe For comparison with true *Microfilaria bancrofti* I could study slides from Houchowfu (Kiangsu Mid China), Gaya (India) and Australia, which I owe to the kindness of Dr Lee, Capt Korke and Dr Heydon

Amongst the specimens from the Malay Archipelago *only those from Java* answered to the descriptions of *Microfilaria bancrofti*, the other ones differed considerably from these descriptions and from the Chinese the Indian and the Australian specimens, I therefore propose to speak of a new species and to name it *Filaria malayi*

II Methods

In only two cases (from Palembang) could I study the living and the freshly dead larvæ vitally' stained according to Rodenwaldt s method with 1 3000 watery azure II solution (Rodenwaldt, 1909) In the other cases I have stained the *thick films strongly with hematoxyline (Delafield) differentiating if necessary* with a 1 per cent solution of hydrochloric acid in alcohol and often using eosine as a counterstain Firstly I have studied the microfilaria by Fulleborn s method of determining the distances of certain fixed anatomical points from the cephalic extremity and expressing these in percentages of the worm s total length (Fulleborn 1913) With the help of Abbe's drawing apparatus the microfilariæ were delineated (magnification 1 500 times) and the anatomical points marked The distances of the latter were then measured and reduced to percentages of the worm's length Fulleborn recommends a curvimeter to measure the length of the curved lines However, I could not get hold of a sufficiently accurate curvimeter, so I resorted to a piece of thin flexible metal wire which was bent along the drawing of the worm the anatomical points were marked on it with Indian ink, the wire stretched along a centimetre measure and the distances read from the latter Most of the measurements however I performed with compasses (I am indebted to Professor Bonne for the recommendation of this method), the two points are fixed at a distance of 1 5 inch and now you let the compasses walk' along the curved line meanwhile counting the number of 'steps , residual distances of less than 1 5 inch are valuated in millimetres By control measurements I satisfied myself that the errors in this method are inferior to ½ mm

In this way the following measurements were taken —

1 Cephalic extremity to nerve ring,
2 ,, ,, , excretory pore,
3 ,, ,, , anal pore,
4 ,, ,, end of nuclear column
5 ,, ,, Total length of specimen

III. Results of Measurements

The results of the measurements of *Microfilaria malayi* together with those of *Filaria bancrofti* which I could gather from literature are represented in the following diagrams. The upper spectrum (as Bonne (1920) wittily called it) represents the measurements of *Microfilaria bancrofti*, the lower one those of *Microfilaria malayi*. Each block represents the mean of 12 to 20 (or sometimes more) specimens from the same person. Details are given in the appended tables. In the upper spectra the data from literature are united with the results of my own measurements, the former being denoted by the authors' names. As may be seen from Diagram 1 the ranges of distances of the nerve rings partly cover each other.

From Diagram 2 it is obvious that the position of the excretory pore also does not form a sufficient characteristic to differentiate both the species.

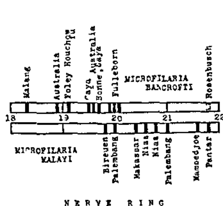


Diagram 1

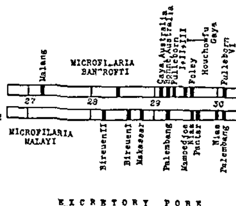


Diagram 2

Diagram 3 proves that the anal pore is situated more anteriorly in *Microfilaria malayi* than in *Microfilaria bancrofti*; however the difference between the Foley series and the Pantar series is very small. Calculation of the probable error proves that it is statistically insignificant.

Diagram 4 represents the distance of the end of the nuclear column as far as it forms a more or less continuous series of nuclei. As we will see later on, in *Microfilaria malayi* there are more posteriorly, still 2 or 3 scattered nuclei. If we call the remnant of the body the tail, it may be stated that in *Microfilaria malayi* the tail is distinctly longer than in *Microfilaria bancrofti*.

Summarizing the measurements teach us that in *Microfilaria malayi* the anal porus is situated more anteriorly and the tail is longer than in *Microfilaria bancrofti*.

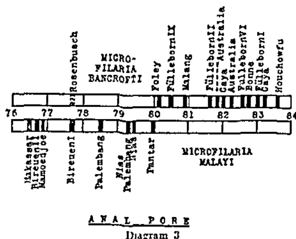


Diagram 3

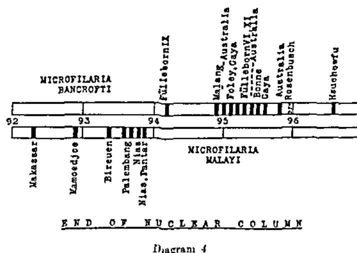


Diagram 4

IV. Other structural differences

It might be considered very doubtful whether the founding of a new species was sufficiently justified, if only the aforesaid differences in measurements existed. However there are other structural differences. Unless otherwise stated these were observed in hematoxyline stained thick blood films. They are:—

1. The *cephalic space* (i. e., the anterior extremity of the body as far as devoid of nuclei) in *Microfilaria malayi* is about twice or one and a half times as long and in *Microfilaria bancrofti* about as long as the breadth of the head (Plate XV, fig. 1). Unfortunately I cannot present you exact data about this characteristic

as most of the measurements were already performed when my attention was drawn to it In two *bancrofti* slides originating from Queensland which Dr Heydon kindly permitted me to examine the cephalic space seems to be somewhat longer than in the other specimens of *Microfilaria bancrofti* However, I am not quite sure whether these slides contained blood films or films of some other body fluid and so it remains dubious whether these specimens invalidate the rule given above, if it is only applied to blood films

2 The *nuclei* in *Microfilaria bancrofti* are neatly arranged so that they can be counted easily In *Microfilaria malayi* most of them form a more or less entangled mass and counting them is only possible with the aid of careful focussing (Plate XV figs 1 and 5)

3 *The position of the genital cells* I could study only in two Palembang cases of *Microfilaria malayi* where I could apply vital staining because the patients resided in the Batavia jail According to Rodenwaldt (1908) confirmed by Fulleborn (1913) the accessory genital cells (G2 G3 and G4) are situated close together just before the anal pore In Plate XV, fig 2, I have copied Fulleborn s drawings In *Microfilaria malayi* the G2 G3 and G4 cells are separated from each other and as well from the anal pore and G3 is situated about half way between the latter structure and the G1 cell Moreover it seems that the G1 cell is bigger in *Microfilaria malayi* than in *Microfilaria bancrofti*

4 In hematoxyline stained specimens *the aspect of the anal pore* is quite different in both species In *Microfilaria bancrofti* it presents itself as a faintly outlined structure situated to the side of the nuclear column without causing a definite break in the latter often difficult to see and sometimes not at all In *Microfilaria malayi* there is a very clear oval gap in the nuclear column sometimes extending over its whole breadth at other times occupying only half the breadth of it but always very obvious (Plate XV fig 3)

In some specimens stained vitally with azure and in some hematoxyline stained specimens the anal pore of *Microfilaria malayi* showed an incision thus presenting a heart shaped form (Plate XV fig 2)

5 The *tail* of *Microfilaria bancrofti* is quite different from that of *Microfilaria malayi* In the former the tail is gradually tapering towards its extremity and conically shaped The form of the tail in *Microfilaria malayi* I could best study in living or freshly dead specimens from the Palembang cases It differs from that of *Microfilaria bancrofti* especially in its posterior half The latter is represented by a tiny thread sometimes a little swollen towards its tip (Plate XV figs 1 and 5) In stained specimens the same structure may be seen, the thread forming the distal half of the tail shows up much thinner than it is during life

Moreover fairly regularly there may be seen two nuclei in the tail of *Microfilaria malayi* one at the end of the thicker part and one at the utmost extremity, usually the former is round, the latter shaped ovally or like an exclamation point The former may be absent, or at least unstained in some specimens Sometimes

there are two nuclei at the extremity either separated by a short space or fused together In Plate XV fig 4 (4) the thread connecting the extreme nucleus with the proximal part of the tail remained unstained

In some specimens the structure of the tail cannot be properly studied because of this part being concealed behind the more anterior parts of the worm or behind the sheath Counterstaining of the hematoxyline stained slides with eosine will give a clearer picture of the tail's structure especially of the protoplasmatic parts

6 The *total length* of *Microfilaria malayi* is inferior to that of *Microfilaria bancrofti* Dr Sardjito kindly determined it in some recently dead malaya larvæ and found it to be 165 to 263μ (mean 234 ± 3 8 μ) in one case and 187 to 265 μ (mean 240 ± 3 4 μ) *in another one, that of Microfilaria bancrofti is generally stated* to be about 300μ According to Fulleborn (1913) the length of recently dead

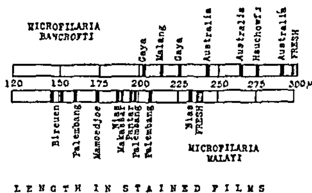


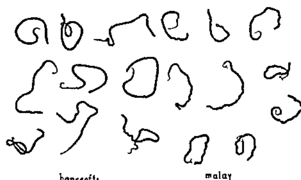
Diagram 5

microfilariæ and even of those that are still living, but that are about to die is greater than that of healthy specimens So, the difference *in length* given here probably is somewhat inferior to the real one The length of worms in stained thick films is so largely dependent on the way in which the films have been prepared that it can hardly be used in differentiating the two species

7 In thick films *Microfilaria malayi* usually does not show the *graceful curves* that according to Manson (1898) are characteristic of *Microfilaria bancrofti* usually in the former species the large curves are complicated by secondary waves However, as Fulleborn (1913) pointed out, the same objection as that raised in the previous paragraph may be made against the use of this point as a differentiating characteristic

8 The *transverse striation* of the cuticle in *bancrofti* is, as a rule, more obvious than that in *malayi* However various *slides of the same species* show a very great range of variation in this respect

Now the question arises Which of these characteristics can best be used for differentiating both species in routine work ? The measurements after Fulleborn are too laborious to be of practical value in routine work Even the mean values of some 20 specimens vary considerably in slides from different origins containing the same species The percentages from the separate larvæ show a still greater range of variation so that measurement of one larva only may be considered to be almost valueless In order to be able rapidly to recognize the proper systematic



Text figure 1

position of each larva separately I would like to recommend the following characteristics —

1 The presence of 2 (1 to 3) scattered nuclei in the tail in *Microfilaria malayi* and the form of the tail

2 The visibility of the anal pore as a clear gap in the nuclear column at about 1 5 of the length of the animal from the posterior extremity in *Microfilaria malayi*

As far as my experience goes both characteristics are very reliable The latter has the advantage of being nearly always visible whereas the tail may be concealed behind the worm s body or behind the sheath

V Distribution of *Filaria malayi*

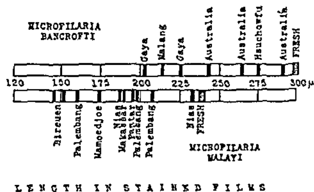
In Text figure 2 those places from which I got slides with *Microfilaria malayi* are marked with a solid circle and Malang where an autochtonous case of filariasis bancrofti occurred with an open circle *Filaria malayi* may be said to be spread throughout the Malay Archipelago It may be noted that from Nias I got 14 slides originating from as many different persons and that all proved to contain *Microfilaria malayi*

With much interest I have read Capt Korke s report on human filariasis in Bihar and Orissa (Korke 1927) especially those parts in which he describes the

there are two nuclei at the extremity either separated by a short space or fused together In Plate XV fig 4 (1) the thread connecting the extreme nucleus with the proximal part of the tail remained unstained

In some specimens the structure of the tail cannot be properly studied because of this part being concealed behind the more anterior parts of the worm or behind the sheath Counterstaining of the hematoxyline stained slides with eosine will give a clearer picture of the tail's structure especially of the protoplasmatic parts

6 The *total length* of *Microfilaria malayi* is inferior to that of *Microfilaria bancrofti* Dr Sardjito kindly determined it in some recently dead malaya larvæ and found it to be 165 to 263μ (mean 231 ± 3 8 μ) in one case and 187 to 265 μ (*mean 240 ± 3 4 μ*) in another one, that of *Microfilaria bancrofti* is generally stated to be about 300μ According to Fulleborn (1913) the length of recently dead



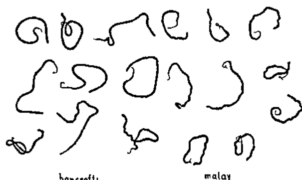
Diagram

microfilariæ and even of those that are still living, but that are about to die is greater than that of healthy specimens So the difference in length given here probably is somewhat inferior to the real one The length of worms in stained thick films is so largely dependent on the way in which the films have been prepared that it can hardly be used in differentiating the two species

7 In thick films *Microfilaria malayi* usually does not show the *graceful curves* that according to Manson (1898) are characteristic of *Microfilaria bancrofti* usually in the former species the large curves are complicated by secondary waves However as Fulleborn (1913) pointed out the same objection as that raised in the previous paragraph may be made against the use of this point as a differentiating characteristic

8 The *transverse striation* of the cuticle in *bancrofti* is, as a rule, more obvious than that in *malayi* However various slides of the same species show a very great range of variation in this respect

Now the question arises Which of these characteristics can best be used for differentiating both species in routine work ? The measurements after Fulleborn are too laborious to be of practical value in routine work Even the mean values of some 20 specimens vary considerably in slides from different origins containing the same species The percentages from the separate larvæ show a still greater range of variation so that measurement of one larva only may be considered to be almost valueless In order to be able rapidly to recognize the proper systematic



Text figure 1

position of each larva separately I would like to recommend the following characteristics —

1 The presence of 2 (1 to 3) scattered nuclei in the tail in *Microfilaria malayi* and the form of the tail

2 The visibility of the anal pore as a clear gap in the nuclear column at about 1/5 of the length of the animal from the posterior extremity in *Microfilaria malayi*

As far as my experience goes both characteristics are very reliable The latter has the advantage of being nearly always visible whereas the tail may be concealed behind the worm s body or behind the sheath

V DISTRIBUTION OF *Filaria malayi*

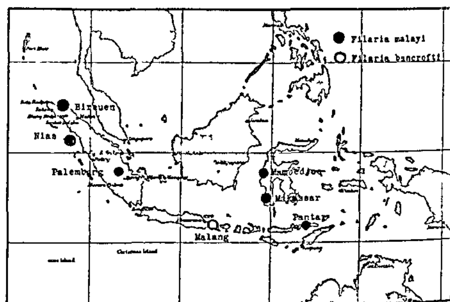
In Text figure 2 those places from which I got slides with *Microfilaria malayi* are marked with a solid circle and Malang where an autochtonous case of filariasis bancrofti occurred with an open circle *Filaria malayi* may be said to be spread throughout the Malay Archipelago It may be noted that from Nias I got 14 slides originating from as many different persons and that all proved to contain *Microfilaria malayi*

With much interest I have read Capt Korke s report on human filariasis in Bihar and Orissa (Korke 1927) especially those parts in which he describes the

microfilaria differing from *Microfilaria bancrofti* and found in Muzaffarpur In this description I find some points suggestive of a relation between these specimens and *Microfilaria malayi*

> Anterior position of the anal pore (at 80 per cent in Nos 48, 49 and 51), 'the tendency of the last tail cell to reach the posterior extremity 'is considerable' (Nos 48 and 51),
>
> Anal pore *distinct*' (No 51, italics are Korke's)

Korke's Fig 12 shows the anal pore as it is often seen in *Microfilaria malayi* Although I cannot come to a definite conclusion as to the nature of the Muzaffarpur microfilariæ it is possible that Korke has observed the same species I saw in Dutch East India



Text figure 2

The microfilaria described by Tanaguchi (1904) occurring in human blood much resembling *Microfilaria bancrofti* but shorter than the latter, may be *Microfilaria malayi* but data are insufficient to establish any more than a supposition

The original description of *Microfilaria youelli* was not availabe in Batavia but to judge from references in textbooks it must be very meagre

Fulleborn (1913) found in slides from East Africa microfilariæ which differed from *Microfilaria bancrofti* and he denoted them as 'sp nov ?' They have the small size in common with *Microfilaria malayi* but otherwise, there is little that pleads in favour of their identity with this species

In 1910 Brochard (1910 1910*a*) described a microfilaria from the Wallis Islands (near Fidji) Two things in his description have especially struck me firstly the anal pore in those larvæ is visible as a clear oval space at about 4/5 of the animal s length and secondly clinical manifestations of filariasis other than elephantiasis were never seen in these islands although some 50 per cent of the population suffered from the latter disease However unlike *Microfilaria malayi* in the Malay Archipelago the microfilaria from the Wallis Islands show no periodicity

So there are some slight indications that the spread of *Filaria malayi* is not restricted to the Malay Archipelago Further research must teach whether there is truth in this supposition

REFERENCES

BONNE C (1920) Onderzoekingen over microfilarien van mensch en hond in Suriname *Ned T jds hr voor Verg Gen* Vol V p 194

BROCHARD V (1910) Recherches sur une microfilaire humaine des Iles Wallis *Bull Soc Path Exot* Vol III p 138

Idem (1910*a*) l Eléphantiasis arabum aux îles Wallis et la theorie filarienne *Ibid* Vol III p 401

FOLEY H (1913) Etudes morphologiques sur les Microfilaires à gaine (*Mf bancrofti* et *Mf diurna*) *Ann Inst Past* Vol XXVII p 50

FULLEBORN F (1913) Beitrage zur Morphologie und Differentialdiagnose der Microfilarien *Arch f Sch ffs u Tr hyg* Vol XVII Beiheft 1

KORKE V T (1927) Observations on Filariasis in some Areas in British India *Ind Jour Med Res* Vol XIV p 71

LICHTENSTEIN A (1927) Filaria onderzoek te Brouen *Gen T jdsch voor Ned Ind e* Vol LXVII p 74

MANSON P (1898) Tropical Diseases London

RODENWALDT E (1908) Die Verteilung der Mikrofilarien im Korper und die Ursachen des Turnus bei Mikrofilaria nocturna und diurna Studien zur Morphologie der Mikrofilarien *Arch f Sch ff u Tr hyg* Vol XII Beiheft 10

ROSENBUSCH F Beitrag zur Erteilung der Mikrofilarien in Argentinien *Centr bl f Bakt* I Orig Vol LXXVIII p 43

TANAGUCHI N (1904) Ueber *Filaria bancrofti* Cobbold *Ibid* Vol XXXV p 49

EXPLANATION OF PLATE XV.

Fig. 1. Cephalic space in *Microfilaria bancrofti* and *Microfilaria malayi*.

,, 2. Position of genital cells in *Microfilaria bancrofti* (after Fülleborn) and *Microfilaria malayi* (original).

,, 3. Anal pore in *Microfilaria bancrofti* and *Microfilaria malayi*.

Figs. 4 and 5. Tail of *Microfilaria bancrofti* and *Microfilaria malayi*.

Fig. 4. Fixed and stained specimens.

,, 5. 'Vitally' stained specimens.

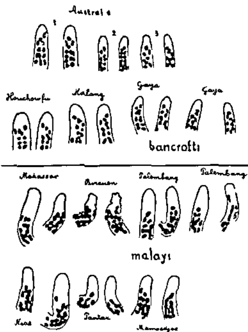
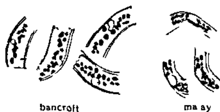


Fig 1



Margashi Malang Delafield

Bireuen
procp



Houchowfu Hæmatoxyline

bancroft

ma ay

Fig 3

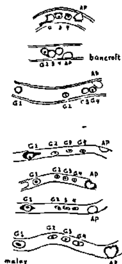


Fig 2



malayi

bancrofti



Fig 4



malayi

Fig 5

TABLE III

Filaria malayi

Pantar

No	N R	F P	A P	Tail
1	20.8	29.0	83.0	7.2
2	22.3	30.1	82.3	3.2
3	23.5	31.7	85.8	3.2
4	21.3	30.1	81.7	4.3
5	23.3	30.7	81.8	5.8
6	24.2	?	83.9	3.2
7	20.5	27.9	79.8	7.4
8	19.1	26.4	77.3	6.7
9	21.4	28.9	77.6	8.5
10	22.3	30.3	83.2	2.2
11	20.9	?	81.2	4.7
12	22.8	30.9	79.3	7.4
13	21.5	29.6	79.8	6.5
14	21.9	29.0	75.9	8.3
15	20.1	28.8	77.0	10.9
16	22.6	32.3	79.2	6.2
17	22.5	31.4	77.5	8.6
18	21.4	28.7	77.9	6.6
19	21.8	?	77.9	7.0
20	21.6	31.4	77.3	9.1
Mean	21.8	29.7	80.0	6.1
P. E	±0.18	±0.78	±0.40	±0.34

TABLE IV.

Filaria malayi

Palembang (?) 1.

No	N R	F P	A P	Tail
1	22.5	32.8	80.0	9.0
2	22.2	31.0	76.8	8.2
3	23.1	32.3	74.7	6.7
4	20.2	29.2	79.8	7.3
5	21.1	32.1	78.8	4.6
6	17.8	26.2	81.7	3.9
7	20.7	28.9	81.4	3.6
8	19.1	27.6	?	8.8
9	17.8	26.5	77.9	8.3
10	20.6	32.8	79.1	5.9
11	26.0	37.2	75.2	8.8
12	21.0	?	?	6.9
Mean	21.0	30.2	78.5	6.3
P. E	±0.43	±0.64	±0.49	±0.37

Table V

Filaria malayi

Palembang 2

No	N P	E P	A P	Tail
1	20 6	30 5	78 8	7 0
2	19 4	28 8	79 3	6 5
3	18 8	28 0	77 2	5 8
4	20 7	29 5	77 6	5 9
5	21 2	30 6	80 3	5 6
6	19 7	29 0	77 3	6 6
7	19 0	30 0	80 6	5 3
8	19 5	27 6	78 5	6 4
9	20 9	29 6	80 5	5 9
10	20 7	29 6	80 0	6 1
11	20 5	30 3	79 8	6 5
12	19 5	29 5	79 6	7 2
13	19 2	28 6	78 2	8 1
14	19 9	?	80 7	5 2
15	19 1	27 3	80 6	5 9
16	19 3	28 8	80 4	7 4
17	20 6	29 6	79 8	4 6
18	19 9	29 5	80 8	8 6
19	21 5	30 4	77 5	7 0
Mean	20 0	29 2	79 3	6 4
P E	±0·11	±0 17	±0 19	±0 15

Table VI

Filaria malayi

Palembang 3

No	N P	E P	A P	Tail
1	19 7	28 5	77 7	8 1
2	18 2	?	81 5	7 2
3	18 3	27 0	78 3	6 7
4	21 6	29 6	82 3	4 4
5	19 9	28 5	77 9	8 3
6	20 0	28 4	80 9	6 2
7	20 7	30 3	80 8	6 6
8	20·5	30 0	78 0	8 3
9	20 6	28 9	80 5	7 1
10	19 7	29 0	?	10 0
11	18 0	25 6	?	8 2
12	17 3	25 3	77 7	7 3
13	19 5	27 3	79 7	5 8
14	21 4	30 4	83 3	4 7
15	19 6	28 7	77 2	6 5
16	19 5	26 9	78 2	7 5
17	18 9	26 5	78 3	7 0
18	19 3	27 0	77 2	7 0
19	18 0	27 5	78 4	6 6
20	20 0	29 6	79 0	5 9
Mean	19 5	28 2	79 2	7 0
P E	±0 17	±0 25	±0 23	±0 19

TABLE VII

Filaria malayi

Nias 1

Patient's No	Mf No	N R	E P	A P	Tail
I	1	19·5	29·0	80·0	6·5
II	1	20·8	29·0	77·3	8·8
III	1	20·5	29·8	78·6	7·4
IV	1	23·1	31·4	80·2	4·0
	2	22·1	31·0	80·5	5·7
V	1	22·3	30·9	79·6	5·4
VI	1	20·8	29·6	80·7	4·5
VII	1	?	?	70·4	6·1
VIII	1	19·7	28·8	81·0	5·3
	2	21·3	30·8	79·8	5·8
	3	20·1	28·8	81·0	7·2
	4	18·9	27·4	80·5	5·8
IX	1	20·6	28·6	76·0	9·4
X	1	20·1	29·4	78·6	4·6
XI	1	?	?	80·8	5·8
XII	1	19·0	27·0	78·4	6·4
	2	23·3	31·0	79·2	5·0
	Mean	20·8	29·6	79·4	6·2
	P E	±0·24	±0·22	±0·25	±0·23

TABLE VIII

Filaria malayi

Nias 2

No	N R	E P	A P	Tail
1	21·8	31·1	80·2	4·[illegible]
2	19·9	29·0	8[illegible]·3	4·0
3	20·5	29·1	80·8	5·5
4	20·9	29·4	77·3	7·8
5	20·6	29·8	80·0	4·9
6	19·0	27·3	78·7	6·6
7	20·1	28·9	77·9	6·8
8	19·8	28·9	80·8	6·0
9	20·3	30·4	78·7	6·4
10	20·0	28·4	76·2	7·4
11	19·8	29·1	79·2	6·0
12	2[illegible]·2	30·7	79·1	5·3
13	22·1	3[illegible]·3	78·3	6·0
14	20·9	29·4	79·4	7·4
Mean	20·6	29·6	79·3	6·1
P E	±0·18	±0·23	±0·24	±0·[illegible]1

TABLE IX

Filaria malayi

Makassar

No	N R	E P	A P	Tail
1	1 0	28 1	78 4	7 1
2	19 4	26 3	77 7	6 8
3	20·8	27 8	70 8	11 5
4	21 2	30·4	81 0	5 4
5	21 4	30 1	?	7 0
6	19 8	29 2	79 0	?
7	20 1	28 8	75 3	6 8
8	20·9	29 7	75 4	8 4
9	21 9	31 1	78 2	5 2
10	20 7	28 6	78 3	6 9
11	20 8	27 9	?	9 1
12	18 1	26 8	74 7	9 8
13	19 2	27 0	?	4 3
14	?	?	75 1	8 8
15	19 7	28 8	?	7 6
16	20 2	29 2	76 2	8 2
17	20 3	28 8	77 5	8 8
18	20 7	31 9	78 3	6 5
19	20·8	29 0	73 6	7 9
20	20 7	27 4	74 3	10 0
Mean	20 4	28 9	76 6	7 7
P E	±0·12	±0 22	±0 40	±0·26

TABLE X

Filaria malayi

Mamoedjoe

No	N R	E P	A P	Tail.
1	19 4	28 0	80 0	5 9
2	22 6	30 0	77 0	8 1
3	23 6	32 5	75 2	6 6
4	24 5	33 9	78 5	7 2
5	20·8	28 2	80 4	7 1
6	24 0	33 7	78 3	6 2
7	20 4	28 4	77 2	8 4
8	22 4	30 8	76 0	5 9
9	21 7	29 5	76 1	6 7
10	21 1	28 9	75 2	7 3
11	20 4	26 1	75 4	7 5
12	18 9	26 8	74 9	10 3
13	21 6	29 8	72 7	8 6
14	20·4	27 0	72 7	9 6
15	21 3	27 0	76 8	8 1
16	21 3	?	75 1	7 1
17	23 0	31 7	79 6	4 3
18	21 2	?	79 5	5 6
19	22 9	30·6	80 2	4 7
20	21 7	28 9	77 0	6 7
Mean	21 7	29 6	76 9	7 1
P E	±0·23	±0·36	±0·34	±0·23

TABLE XI

Filaria bancrofti

Townsville European No. 1.

No	N R	E P	A P	Tail
1	19.0	30.3	81.5	3.9
2	18.4	29.0	?	4.1
3	19.2	28.7	80.6	4.2
4	18.6	28.4	83.6	3.7
5	18.7	28.0	81.6	3.4
6	19.4	26.7	82.0	4.6
7	19.5	27.3	81.4	5.1
8	18.5	27.0	?	3.4
9	19.2	29.0	?	4.1
10	17.7	27.9	80.3	5.8
11	19.6	30.0	81.4	3.4
12	17.6	27.2	?	5.0
Mean	18.9	28.4	81.9	4.2
P. E	±0.14	±0.22	±0.35	±0.15

TABLE XII

Filaria bancrofti

Townsville European No 2

No	N R	E P	A P	Tail
1	19.1	28.7	82.9	5.0
2	?	28.8	83.0	3.7
3	17.3	25.0	8 5	4.4
4	21.8	31.4	83.7	3.9
5	22.3	31.5	81.7	5.6
6	20.8	30.1	82.4	6.7
7	17.4	27.3	79.0	6.1
8	19.3	28.3	82.3	6.2
9	20.8	30.3	81.4	4.4
10	19.8	30.8	80.8	6.8
11	17.9	26.5	81.2	6.9
12	19.2	29.5	?	4.5
13	19.5	28.7	81.2	5.2
14	20.4	30.0	83.2	3.8
15	19.2	28.2	80.4	6.0
16	19.5	29.9	83.8	4.7
17	20.1	29.6	81.5	5.3
18	19.2	28.9	84.3	4.1
19	19.9	30.1	82.8	4.8
20	19.8	30.0	83.7	3.8
Mean	19.6	29.2	82.2	5.0
P E	±0.19	±0.21	±0.21	±0.10

Table XIII.

Filaria bancrofti.

Queensland—Native.

No	N R	E P	A P	Tail
1	?	29 5	80 6	5 7
2	20 4	28 7	84 1	5 0
3	18 6	28 9	?	4 6
4	17 6	28 7	84 0	4 7
5	19 2	28 5	83 2	4 2
6	21 5	29 5	82 7	4 6
7	17 9	27 8	81 2	4 6
8	22 3	28 8	81 7	4 4
9	18 8	28 8	83 0	4 4
10	?	29 6	82 7	4 0
11	18 6	30 2	79 8	6 0
12	20 5	29 8	82 5	3 6
13	19 5	29 1	82 2	4 7
14	19 6	30 9	85 3	3 8
15	?	28 3	81 9	3 6
16	22 5	29 4	81 9	4 3
17	21 4	30 9	82 8	4 4
18	?	28 7	80 3	4 3
19	18 7	29 1	81 4	5 4
20	16 3	27 3	80 6	5 8
Mean	19 6	29 1	82 2	4 6
P. E	±0 28	±0 14	±0 22	±0 10

Table XIV.

Filaria bancrofti

Houchowfu.

No	N R	E P	A P	Tail
1	19 6	29 2	83 2	3 8
2	19 3	30 1	83 6	4 0
3	18 6	30 0	83 8	3 6
4	19 0	29 3	85 2	3 0
5	18 5	28 1	87 1	3 7
6	17 9	28 2	83 2	3 2
7	19 5	30 0	82 4	3 6
8	18 9	30 0	83 8	3 1
9	18 6	29 2	83 8	2 7
10	19 2	30 0	83 5	3 4
11	20 2	30 2	82 8	4 1
12	19 1	29 0	82 4	3 0
13	18 4	28 3	81 8	4 7
14	19 9	30 6	85 5	2 3
15	18 8	29 1	83 0	3·0
16	20 4	30 5	85 2	2 4
17	19 2	30 3	78 9	4 5
18	19 5	29 3	85 2	2 8
Mean	19 1	29 5	83 6	3 4
P E	±0 07	±0 13	±0 28	±0 11

TABLE XV

Filaria bancrofti

Malang

[illegible]	N R	E P	A P	Tail
[illegible]	18 2	?	80·8	5 2
[illegible]	18 9	28 8	81 9	4 6
[illegible]	17 8	26 2	80·3	6·9
[illegible]	17 8	25 4	80·0	5 3
[illegible]	17 5	27 3	81 4	4 9
[illegible]	18 7	26 8	81 7	4·0
[illegible]	18 5	28 4	83·9	4 4
[illegible]	19 2	29·0	?	5 7
[illegible]	18 2	25 9	81 5	4 2
[illegible]	17 4	25 7	81 5	4 9
[illegible]	18 5	28 1	80 6	4 2
[illegible]	18 6	27 4	82 0	4 1
[illegible]	18 1	25 5	82 9	4 7
[illegible]	19 2	28 7	?	5 2
[illegible]	18 3	28 4	81 7	4 6
[illegible]	17 9	26 8	80 2	6 3
[illegible]	19 1	28 9	79 7	6 0
[illegible]	19 1	28 2	80 7	5 2
[illegible]	18 4	28 2	80 2	5 0
[illegible]	16 9	25 6	78 8	5 2
[illegible]	18·0	27 3	81 1	4 8
[illegible]	17 5	26 8	80 4	5 4
[illegible]	18 1	27 7	79 8	5 4
[illegible]	°0 2	31 2	79 8	5 1
[illegible]	18 1	27 4	80 4	5 6
	18 3	27 2	80 9	5 1
	±0 09	±0 10	±0 16	±0 10

TABLE XVI

Filaria bancrofti

Gaya 1

No	N R	E P	A P	Tail
1	19 7	28 6	80 9	4 7
2	19 4	29 0	83 3	4 1
3	24 7	30·4	81 8	5 5
4	19 1	29 4	79 5	5 3
5	18 2	28 7	8_ 2	4 6
6	18 9	29 6	8° 4	4 7
7	°0·1	30 8	83 8	3 9
8	19 2	29 0	81 5	5 1
9	20·4	30 5	82 4	4 7
10	18 6	27 3	82 7	4 0
11	20 4	31 5	82 8	7 2
12	18 0	27 2	78 9	6 2
13	21 4	31 3	82 4	4 3
14	19 3	29 8	83 3	4 8
15	18 8	28 3	81 2	4 4
16	19 0	29 6	82 7	5 2
17	18 1	27 2	8° 0	4 2
18	19 1	29 7	?	5 4
19	19 9	29 5	82 4	3 8
20	21 6	3 4	82 2	4 5
Mean	19 7	29 5	82 0	4 8
P E	±0 22	±0 °2	±0 19	±0 12

TABLE XVII

Filaria bancrofti

Gaya 2

No	N R	E P	A P	Tail
1	18 5	28 0	81 5	4 9
2	20 6	30 9	82 1	4 6
3	19 9	29 1	84 0	2 6
4	20 4	30 3	80 9	5 6
5	19 2	30·0	82 8	4 2
6	20 7	30 7	83 8	3 7
7	19 5	30 0	82 7	5 5
8	19 9	28 8	82 4	4 8
9	20 0	28 1	84 2	4 2
10	20 5	28 6	81 7	5 9
11	18 9	28 2	80 9	6 2
12	18 2	28 8	82 4	4 1
13	18 1	26 1	70 9	3 8
14	18 4	29 0	82 6	4 9
15	21 1	30 7	84 0	3 4
16	18 9	28 8	83 4	3 6
17	18 0	28 0	83 9	4 0
18	20 8	30 1	84 2	3 9
19	19 5	28 9	83 5	3 3
20	19 9	28 9	80 5	4 1
Mean	19 5	29 1	82 6	4 4
P E	±0 15	±0 18	±0 20	±0 14

TABLE XVIII

Measurements of Mf bancrofti

Recorded in literature

Author	Origin	Nerve Ring Mean P E	Excretory Pore Mean P E	Anal Pore Mean P E	Tail Mean P E
Bonne	Surinam	19 7±0 14	29 2±0 14	82 6±0 14	4 5±0 11
Foley	Africa	19 1±0 15	29 6±0 20	80 1±0 09	4 9±0 06
Fülleborn I+II+III	St Thomé	19 1±0·09	29 3±0 14	82 0±0 26	
Id IV	*New Guinea.*	18 3±0 33	28 6±0 40	83 5±0 23	
Id VI	East Africa	19 9±0 11	30 1±0 18	82 6±0 30	4 7±0 10
Id VII+VIII	,	19 5±0 20	29 1±0 33	83 1±0 41	
Id IX	Philippines	19 6±0 18	29 9±0 1[illegible]	80 5±0 27	5 8±0 17
Id X+XI	Samoa	19 7±0 18	29 1±0 20	82 7±0 12	4 7±0 21
Rosenbusch *	*Argentine*	*21 8*	*30 2*	77 7	4 0

* Details not stated

FILARIAL INFECTION AND DISEASES DUE TO *FILARIA BANCROFTI*

BY

S SUNDAR RAO,

Darbhanga Scholar Calcutta School of Tropical Medicine and Hygiene

THE object of this short paper is to give essentially an indication of the work that is being done in the Calcutta School of Tropical Medicine and Hygiene on filariasis The investigations which were started in May 1921 comprehend a survey of the distribution of filariasis in India a pathological study of the diseases due to *Filaria bancrofti* observations on the treatment of these diseases and mosquito infection The work that has been done falls therefore into four main divisions —

(1) Field investigation
(2) Pathological examination
(3) Treatment
(4) Natural and experimental mosquito infection

(1) FIELD INVESTIGATION

During the last six years a large area has been surveyed in detail for the prevalence of filarial infection This included the Coromandel Coast in the Madras Presidency the more important tracts in the province of Bihar and Orissa by far the larger portion of Central and Southern Bengal including Calcutta and certain hilly regions in the United and Central Provinces

The local investigation consisted of a filariasis survey an entomological survey, and a clinical survey The filariasis survey was carried out in the following manner —Every district centre was visited and the microfilaria rate was obtained by the examination of a large number of persons This was rendered possible by the authorities in charge of the district jails affording every possible facility for the *examination of those prisoners who were natives of the district in question* In some places the examination of the jail population was supplemented by the examination of the patients at the local hospitals and other public institutions Blood smears taken at night were also received by post from several other places and afforded further material of work

In the large area thus surveyed, the degree of infection was found to be very varying.

An examination of the following Table leads to the recognition of three distinctly marked areas which differ from each other in their microfilarial rate These are the non endemic areas where no filarial infection was found in the blood, the endemic areas with a microfilarial rate of under 10 per cent and the endemic areas with a microfilarial rate of over 10 per cent

TABLE

Showing Results of a Filariasis Survey

Areas	Number Examined	Number Positive	Percentage
Bengal			
Calcutta	841	83	10
Alipore	457	47	10 3
Howrah	164	10	6 1
Hooghly	60	7	12
Midnapore	1 491	122	8 2
Bankura	50	7	14
Burdwan	50	4	8
*Suri	117	5	4 3
*Krishnagar	139	3	2 1
*Jessore	228	4	1 7
*Chittagong	107	0	0
*Dacca	300	15	5
Pabna	85	3	3 5
*Berhampore	150	6	4
*Rajshahi	200	1	0 5
Dinajpur	386	37	9 6
*Jalpaiguri	200	0	0
*Darjeeling	12	0	0
Assam			
*Srimangal	75	0	0

* Blood smears received by post

TABLE—*concld*

Areas	Number Examined	Number Positive	Percentage
Bihar & Orissa			
Cuttack (Total)	2 000	240	12
(Jail)	492	125	25
Balasore	40	3	7
Purulia	358	23	6.4
Ranchi	90	0	0
Hazaribagh	63	0	0
Gaya	93	14	15
United Provinces			
Almora	64	0	0
Bhowali	36	0	0
Bhim Tal	10	0	0
Central Provinces			
Saugor	100	0	0
Madras			
Berhampore	35	3	9
Vizagapatam	81	10	12
Kristna	38	3	7.9
Rajahmundry	150	15	10
Guntur	54	1	2
Nellore	37	1	3
Madras	61	6	10
*Vellore	25	0	0
*Mangalore	79	1	1.2

*Blood smears received by post

Among the non endemic areas may be mentioned Almora Darjeeling and Ranchi most of which are at an elevation of above 2 000 *feet above the sea level* while most of the highly endemic areas are either on the sea coast as in the case of Vizagapatam and Madras or in densely populated and low lying districts with poor sanitation such as Gaya Hooghly and Cuttack

One of the most heavily infected towns is Cuttack with a microfilarial rate of 25 per cent with respect of the jail population and 12 per cent on the total population In Calcutta amongst the patients admitted into the Carmichael *Hospital for Tropical Diseases for diseases other than filariasis* out of a total of 841 examined 83 patients had microfilariæ in their blood giving a percentage of 10 The distribution of the infection in the city *of Calcutta is general with a* tendency to a higher percentage in the congested localities of north Calcutta and Kidderpore

It may be stated generally that certain optimum conditions of temperature and humidity favour the prevalence of filariasis Observations in India confirm the general finding that the endemic areas are situated either along the sea coast or in the neighbourhood of rivers or tanks Similarly, observations taken at two adjacent places *Purulia and Usmanpur barely five miles apart go to show that the* endemicity of filariasis also depends upon the presence of the human carrier

Simultaneously with the examination for filarial infection in an area the mosquito fauna of that area was worked out and it is interesting to note that *Culex fatigans* the chief efficient intermediary host of *Filaria bancrofti* in India is found to be present in all the areas surveyed endemic and non endemic

A clinical survey of the local area completing the field work afforded valuable indications It was found that apparently healthy people have been harbouring filarial parasites for a long time without any indication of their presence and that the poorer classes are *more susceptible to filarial diseases perhaps owing to want* of proper housing and nourishment The commonest phases of the diseases met with in the endemic areas are lymphangitis lymphadenitis and elephantiasis, chyluria and abscess are common though not so frequently met with as those mentioned above Lymphatic varix lymph scrotum and chylocele are much less frequent Filarial fever is the most general manifestation of the infection In Calcutta out of 1 308 cases admitted at the out door for filariasis 936 cases sought treatment for lymphangitis 314 cases *for treatment of elephantiasis only* and only 58 for other manifestations of the infection such as chyluria abscess or hydrocele *The clinical signs and symptoms of filarial* fever were typical

As regards the age incidence of filarial diseases out of 822 cases 3 were below the age of 10 142 between 11 and 20 247 between 21 and 30 227 between 31 and 40 143 between 41 and 50 and 60 between 51 and 60 and it would appear that lymphatic obstruction occurs about the age of 15 and reaches its height between 20 and 30

Filarial diseases were found to be common in all the races and both the sexes

In 92 7 per cent of the cases of elephantiasis met with the lower limbs were affected while the scrotum was affected in 5 3 per cent of the cases Filarial affections of the hands (1 7 per cent) and mammæ (0 3 per cent) were rare

In a scrutiny of 100 cases of elephantiasis of the lower limb it was found that in 15 cases both the legs were involved in 46 cases the right leg and 39 the left

(2) Pathological Examination

This was chiefly directed to the study of the pathogenesis of the diseases and of the role of secondary organisms A study of changes in the blood condition in filarial infection and diseases was made and the possibility of the presence of septic focus being present in the body of cases of filarial diseases was investigated as many of these exhibit signs and symptoms of streptococcic or staphylococcic infection

The results of the pathological study are being embodied in an article now in the course of preparation by Lieut Col H W Acton I M S

The average eosinophilia count in filarial infection and diseases excluding other helminthic infection was found to be 8 per cent the maximum being 49 per cent and the minimum 1 per cent

The presence of a septic focus in cases of filarial diseases was recognized in 193 out of 282 cases investigated along the course of alimentary canal In the majority of the cases the patients were suffering from pyorrhœa or dental caries and infections of pharynx and tonsils In a few the focus was in the lungs or intestines

(3) Treatment

During the period under review several drugs were tried in filarial infection and diseases and it was found that none of these gave any permanent benefit The following is a list of drugs tried Antimony S A T 2 per cent 471 Urea stibamine Novo stiburea Arsenic Somnin Hectine Sod Cacodylas Neo Salvarsan Novarsinobillon Sulfarsenol Myo salvarsan Silver Salvarsan Tryparsamide Hydrarg perchloride 1 in 1000 Emetine Hydrochloride Iodine Solution

Of these antimony in its various forms was given in 35 cases A temporary decrease in the microfilariæ in the blood was noticed during treatment with slight diminution in lymphangitis and œdema but antimony had no influence in checking the attacks Similarly several compounds of arsenic were given intramuscularly and intravenously They had no lasting effect in reducing the number of microfilariæ in the blood These compounds of arsenic temporarily checked the recurring attack of lymphangitis and in this respect were better than the antimony compounds

But much benefit was to be got out of mixed vaccines of strepto and staphylo cocci obtained from skin lesions in checking the attacks of lymphangitis and reducing œdema The injections were given intradermally in graduated doses up to maximum of 1 c c which is equal to 100 million streptococci and 1 000 million staphylococci

(4) Natural and Experimental Mosquito Infection

In Cuttack, Khargpur and Calcutta a very large number of 'wild' mosquitoes were dissected for embryos of filariæ, with the result that as many as 10 per cent were found to be infected at Cuttack and only 2 5 per cent each at Khargpur and Calcutta Work done in Cuttack throws some light on the variations in the infectivity rate of *Culex fatigans* during different periods of the year Here, in July the infection rate was 3 per cent, in August, September and October it was 7 5 per cent and in November and December it was found to be 12 5 per cent This is an interesting observation showing that the natural infection is greatest during the winter months

Laboratory bred *Culex fatigans* were fed at night time on persons having micro filariæ in their blood and were dissected to find out the infection rate under laboratory conditions in different seasons An examination of the mosquitoes showing embryos undergoing development after experimental feeds at the different seasons during the same year at Cuttack showed the following percentage of infection May and June 13 per cent, August, September and October 34 per cent, December and January 78 per cent

These figures show that the highest infection rate of *Culex fatigans*, fed on cases, is during the months of November and December which bears out the observations made on 'wild' mosquitoes where also the infection rate reaches its maximum during the same period

Observations were also made on *the time taken for the microfilariæ to develop in* the body of the mosquito There appears to be a close relation between the tempera ture and the time taken for development as in August with an average maximum temperature of *93° F, it took only 14 days to reach the proboscis, while in December* with an average maximum temperature of 80° F, it took 22 days The minimum temperatures were 86° F and 55° F in August and December respectively, while the humidity was *90 and 85 per cent*

As is well known, this work has been rendered possible by the munificent endowment of the Maharajadhiraj of Darbhanga, and the little that has now been achieved *is entirely* due to the very able and sympathetic management and guidance bestowed upon it by Lieut Col J W D Megaw, C I E, V H S, I M S, Director, and Lieut-Col H W Acton, I M S, Professor of Bacteriology, Pathology and Helminthology, Calcutta School of Tropical Medicine, to whom the writer is much indebted.

PREVALENCE OF FILARIASIS IN SOME AREAS IN BRITISH INDIA

BY

VISHNU T KORKE, F R C P (Edin) D T M (L'pool)
Central Research Institute, Kasauli

I Introduction

The investigation into filariasis was undertaken in the agricultural, hospital, police and convict groups of population of the district areas in Bihar and Orissa The areas investigated were Shahabad, Palamau Gaya, Hazaribagh, Muzaffarpur and Cuttack

The preliminary object of the inquiry was first to make rapid survey of the type species prevalent in the endemic areas in relation to the physical characters of the district and secondly to observe the early clinical features associated with such species

II Material and Technique

The cases selected for examination were males The leading signs and symptoms were of the following nature —

(i) A long duration

(ii) History of fever

(iii) Enlargements	Glandular Genital Terminal	Upper and lower extremities
(iv) Effusions	Nature	Permanent Fugitive

In all cases the peripheral blood in a quantity of about 20 c mm was examined The time for taking the blood was either during the day night or both

The cases in which microfilariæ were found were classed as 'positive,' the rest as 'negative'

The blood preparations were stained in a weak watery solution of Giemsa They were also stained in Delafield's hæmatoxylin (time 24 hours in both cases)

Camera lucida drawings of the parasites found in each area were made as a routine method

The morphology of the parasites was studied from the standpoint of measurements of the different anatomical fixed points, as given by Fullebom (Korke, 1927)

III Results Obtained in Field Studies

The physical characters of the district areas and the groups of population investigated were as follows —

Area Shahabad 4 400 square miles occupies the angle formed by the junction of the Sone and the Ganges Physical divisions, 3 Northern region an extensive low lying alluvial plain 550 square miles and subject to inundations by the Ganges This tract forms a principal wheat growing area of Bihar Middle region 3 000 square miles a very fertile area extensively irrigated highly cultivated, and densely populated Southern region 800 square miles an undulating table land, about 1,500 feet above the sea level , the forest area is scattered along this tract of land Soil a species of tenacious clay intermixed with coarse sand Mean temperature 62° F to 90° F Mean annual rainfall 46 inches Density per square mile northern region 890 persons , southern division 181 persons , district as a whole 449 persons Staple crop rice

Total cases 291 , positive, 25 , incidence, 8 5 per cent Hospital cases 78 positive 6 Convict cases 107 positive, 10 Police cases 60 positive 5 Agriculturist cases 49, positive 4

Blood examinations 334 , night 293, positive, 25 , day 21 positive, 6 combined 20 positive 6 Type species *F bancrofti*

Area Gaya 5,000 square miles forms the southern portion of the Patna division Physical divisions 2 Northern region an extensive alluvial plain 3 000 square miles, a principal rice growing area The north and north western portions are extensively irrigated highly cultivated and densely populated Southern region an elevated area and an undulating table land, about 2 000 square miles and 1,200 feet above the sea level A large tract of this area is a forest area Soil species of clay intermixed with varying amounts of sand and could be classified into four groups Temperature 93° F to 105° F Rainfall 45 inches Density northern region 666 persons , southern region, 278 , district as a whole, 437 persons Staple crop, rice

Total cases 9 , positive 1 , incidence 11 per cent Hospital cases 2 negative 2 *Agricultural cases 7 , positive,* 1

Blood examinations 9 night in all cases Type species *F bancrofti*

Area Palamau 5 000 square miles forms the northern corner of the Chota Nagpur division Physical divisions Area in the shape of a parallelogram intersected by a series of parallel ranges of hills running east and west through which the river Koil passes northwards to join the Sone The arable land about 900 square miles average elevation of the district 1,200 feet Forest area 3 200 square miles Soil a species of hard stiff clay, extremely retentive of moisture Temperature 81° F to 93° F Rainfall 45 inches Density 149 persons Staple crop rice

Total cases 17 positive, 3 , incidence 17 6 per cent Hospital cases 12 positive, 1 , convict, 3, positive, 0 , agricultural, 2 positive 2

Blood examinations 23 night 17 positive 3 day 3 positive combined 3 positive 2 Type species *F bancrofti*

Area Hazaribagh 7 000 square miles forms the north eastern portion of the Chota Nagpur division Physical divisions 3 A lower plateau 800 feet above the level of the Gaya plain a higher plateau 200 square miles with an average height of 2 000 feet and the Damodar basin Forest area about 5 000 square miles Soil varieties of soils have been classified under 6 groups Temperature 40° F to 107° F average 75° F Rainfall 51 inches Density district as a whole 186 persons Staple crop rice

Total cases convict only 32 positive 2 incidence 6 5 per cent

Blood examinations 84 night 31 positive 2 day 27 positive nil combined 26 positive nil Type species *F bancrofti*

Area Muzaffarpur 3 000 square miles between the Ganges on the south and Nepal frontier on the north Physical divisions 3 Southern tract along the Ganges the richest and most fertile Middle tract an area of low lying land subject to inundations by the large rivers which intersect the district Northern tract about 1 500 square miles a great marshy plain to the Nepal frontier The whole district is well watered Soil sand and clay intermixed in varying proportions and could be divided into 4 groups Eighty per cent of total area cultivated Temperature between 73° F to 97° F Rainfall 48 inches Density 908 persons for the district Staple crop rice

Total cases 32 Hospital cases 8 all negative Convict 24 positive 2 incidence 6 per cent

Blood examinations 32 all night examinations positive 2 Type species *F bancrofti*

Area Cuttack 3 600 square miles a central district of Orissa Physical divisions 3 The first a marshy woodland strip 90 square miles a littoral tract facing the Bay of Bengal The second is a cultivated alluvial plain and the third is a submontane broken hilly region forming the western boundary of the district The most conspicuous feature in the general aspect of the district is its system of rivers Forest area about 90 square miles There are no extensive forest areas Soil sand and clay intermixed in varying proportions Temperature varies between 69° F 102° F and 116° F according to seasons Rainfall 60 inches Density 1 084 per cultivated square mile Staple crop rice irrigation is far less essential owing to ample supply of rainfall

Total cases agricultural group 35 positive 6 incidence 17 per cent

Blood examination 41 night 35 positive 6 day 3 positive nil combined 3 positive nil Type species *F bancrofti*

The results show that 7 per cent were positive in the hospital group 8 2 *per cent* in the police group 8 5 per cent in the convict group and 13 8 per cent in the agricultural group of population The type species was *F bancrofti*

The total cases were divided into two classes —(*a*) those showing clinical signs and *symptoms total* cases 169, positive 24, and (*b*) those which did not, cases, 250 positive 15

Cases showing clinical signs and symptoms show the following analysis —

Duration of illness ranged between 1/12 to 25 years, history of fever 61 5 per cent permanent œdemas 50 per cent, glandular enlargements, 45 per cent, genital enlargements 20 per cent, terminal enlargements, 14 2 per cent, and cases showing fugitive œdemas 3 5 per cent

In cases not showing *any clinical signs and symptoms* 6 *per cent showed* microfilariæ in the blood

Out of 54 blood examinations made during the day time 11 per cent showed microfilariæ in the blood

IV Discussion of Results

I have shown previously that after eliminating by differential diagnosis certain *obvious affections of the genitals, affections pertaining to the generative organs* and glandular enlargements are usually filarial in origin in an endemic area On morphological grounds the identification of *F bancrofti* as are found in the endemic areas of Bihar and Orissa has also been shown (Korke, 1927)

The point to which emphasis may be given here is the usefulness of the investigation into *the normal cases and diurnal blood examinations for F bancrofti* in the endemic areas These points may throw light on the nature of very early infections and on the species of vectors which are possibly diurnal in habit

The evidence, although slender, supports the view that the agricultural classes of population seem to be more infected in a lowland area like Cuttack whose surface *approaches to the level of the sea, than an upland area*

V Conclusions

(1) There is a considerable prevalence of filariasis in the different groups of population in the areas of Bihar and Orissa

(2) Fourteen per cent of cases showed microfilariæ in the peripheral blood where filariasis was suspected on clinical grounds and 6 per cent in cases where it was not suspected

(3) The microfilariæ were detected most readily during the night time Eleven per cent of cases showed them during the day time

(4) The notable clinical features were affections of the glands (chiefly inguinal) and the genitals

(5) The predominant species was *Microfilaria bancrofti*

(6) It is suggested that the blood examinations may be made both during the day and night time, and that the normal individuals may also be investigated along with the suspected cases in an endemic area in order to arrive at a conclusion regarding the filarial situation of the area

REFERENCES

Bengal District Gazetteers — Cuttack, Gaya, Hazaribagh, Muzaffarpur Palamau and Shahabad Bengal Sectt Book Depot

Korke (1927) — *Ind Jour Med Res*, Vol XIV, No 3

DISCUSSION

Major H H King, I M S (Madras) (1) Objected in the interests of nomenclature to the name *F malayi* given to the filaria described by Col Brug, because, Malaya being a country and not an individual, the name should be either *F malayensis* or *F malayæ* as Col Brug preferred

(2) While Dr Korke's and Dr Sundar Rao s surveys were over wide tracts of country, the King Institute in Madras was making a survey of quite a different nature, an intensive survey of a small area in the town of Saidapet Dissections of *Culex fatigans* found in houses showed a high infected rate of 35 per cent and the question arose (as raised previously by Major Sinton I M S) were these all *F bancrofti* The solution which the Institute was attempting was to study mature filaria derived from infection experiments on crows, dogs etc Experimental filariasis transmission on the filaria of a lizard had been done by Dr Pandit and other workers which he would leave to Dr Pandit to describe

(3) The drugs so far tried in treatment seemed to be all drugs that would be absorbed by the blood stream He suggested that a fatty drug in an emulsion or as an oil, which would be parasiticidal to filaria larvæ and adults should be tried The tissues peripheral to the sites where these worms are found would be infiltrated or injected with this drug which by its nature would remain in the lymphatics and travel up there and thus damage or kill the worms Naturally the early stages before lymphatic obstruction occurred would be best for treatment

Dr C G Pandit (Madras) There is a high incidence of filarial infection in mosquitoes caught in a house, but there is no correlation between the infected mosquito rate and the filarial rate in the inmates of the house While doing a filarial survey some difficulty arises in the diagnosis of filarial conditions in an endemic locality an abscess need not always be filarial The difficulty is great when dealing with early cases which begin with slight swelling of the extremities It appears from the work done in the Institute that the complement fixation test can be utilized to diagnose such cases A filaria survey of animals infected in nature is of some importance Work done in the King Institute during the last year or so has shown that even in animals the same kind of patchy distribution with infection localized to the vicinity of water, etc , has occurred Transmission has been successful by causing infected mosquitoes to bite healthy animals This question is being investigated

Dr T S Tirumurti (Madras) Calling *F malayi* by a place name is objectionable It would be better to call it *F brugi*

The theory of focal sepsis is unnecessary to explain certain filarial manifestations We can find focal sepsis in all tropical diseases but these diseases are not attributed to focal sepsis The parts where lymphangitis occurs are those subject to irritation There are strepto and staphylococci in the skin which give rise to the lymphangitis in these cases Jail statistics are not reliable sources of information Prisoners come

from all parts of the Presidency and also *from all parts of India* *Even statistics* discovered from microscopical examinations are bound to be unsatisfactory, but are the best which can be obtained under the circumstances It is well known that the same patient does not show microfilariæ, even in thick smears every night In an endemic area filaria as a cause of enlargement of glands should be remembered but all the other common causes should not be forgotten, just as every fever in an endemic area is not filarial in origin Treatment can only be of use to prevent *recurrences of fever, lymphangitis and other filarial manifestations, but the results* of filariasis, such as elephantiasis, etc , have come to stay It is impossible to treat results which have become permanent

Dr S C Basu (Bihar and Orissa) I do not believe that the clinical manifestations of filariasis are due to streptococcal infection as stated by Dr Rao Filarial fever is a periodic fever which lasts for some 36 to 48 hours and comes down by crisis The treatment of the clinical manifestations of filariasis is very unsatisfactory We have tried various drugs viz Bayer '205,' Heydens '471,' Rivanol, formalin and iodine salvarsan, sodium antimony tartrate in 2 per cent solution, and vaccines We have found all these drugs are very unsatisfactory except sodium antimony tartrate *and salvarsan In some cases sodium antimony tartrate solution and in others salvarsan* give excellent results One hundred and twenty two c cs of sodium antimony tartrate in a 2 per cent solution given by injection can extirpate microfilariæ from the blood One per cent iodine solution kills adult parasites in the dog where these parasites are found in the subcutaneous tissues, but in human beings we do not know where the adult parasites live so that we cannot kill them

Some patients, treated with vaccines (strepto and staphylococcal) in the School of *Tropical Medicine in Calcutta have had a recurrence of the disease a few months* afterwards

Col S L Brug (Netherlands East Indies) replied As regards the name of the new filaria I must remark that it has already been printed in a preliminary note in Dutch so it can only be altered if the name is pre occupied or if the name is grammatically incorrect I am no expert in Latin and I dare not decide whether 'malayi' must be amended into 'malayensis The name *Aëdes malayi* for a Culicid has existed since 1908 without ever being objected to for grammatical reasons

Dr Y T Korke (B India) Pointed out in reply to Major King's and Dr Tirumurti s observations about naming of the spp of *F malayi* by Col Brug that *if the Latin* terminations were not correct, they could be rectified in accordance with the laws of zoological nomenclature, but as regards naming of the species, the author was privileged to give the species the name of his own choice

Epidemiological observations in large areas provide a greater scope for observation These could be finally focussed down to a particular type of observation the nature of which had been described by Dr Pandit Scientific information of any value which may ultimately throw light on the true filarial situation of any area should not be *neglected*

SECTION V.

DISEASES OF NUTRITION.

DISEASES OF FAULTY NUTRITION.

BY

LIEUT COL R McCARRISON, C I E, M D, D Sc, F R C P, I M S
Director, Deficiency Diseases Inquiry, Indian Research Fund Association

MORE than 2 000 years ago Hippocrates referring to the great difference which kind of bread makes to the human body, wrote as follows —

MONDAY DEC 11 A 1 PM

'Whoever pays no attention to these things, or paying attention, does not comprehend them, how can he understand the diseases which befall a man? For by every one of these things a man is affected and changed this way and that and the whole of his life is subjected to them, whether in health convalescence or disease Nothing else, then, can be more important or more necessary to know than these things'

'Whoever does not know what effect these things produce upon a man cannot know the consequences which result from them nor how to apply them'

'Wherefore it appears to me necessary to every physician to be skilled in nature, and to strive to know if he would wish to perform his duties what man is in relation to the articles of food and drink, and to his other occupations and what are the effects of each of them to every one'

It is strange that, although these words were written so long ago it is only within the last quarter of a century that we have begun to pay attention to 'what man is in relation to the articles of food and drink' to 'know what effect these things produce upon a man,' and 'to understand the diseases which befall a man' in consequence of them

In the time at my disposal I can do no more than give a very brief outline of the present state of knowledge of the nutritional or, as I prefer to call them, the malnutritional diseases I shall not, therefore, concern myself with morbid states which result from the ingestion of food in insufficient quantity, nor with

those which may be associated with over eating but will confine myself to ailments whose genesis is *directly or indirectly dependent upon the improper quality or the improper balance of food ingested in sufficient quantity*

Since the functions of food are to rebuild the living tissues to supply energy and to preserve a proper medium in which the biochemical processes of the body can take place it follows that derangements of nutrition—and therefore of health—must result if the food ingested fails adequately to subserve these functions Then the architecture of the living tissues becomes imperfect transformation of energy in the body—the most fundamental of all considerations in nutrition—becomes deranged and metabolic processes become disordered with the consequent pro duction of abnormal or it may be of toxic metabolites The failure of food to subserve the e *functions may be brought about in a number of ways but the one* which chiefly concerns us here is the insufficient provision in the diet of one or other or all of three of its essential constituents suitable protein inorganic salts and vitamins Foods which are unsatisfactory in these regards give rise to sub optimal or to sub normal states of health or even to actual disease the character and the severity of which depend upon the nature and degree of the food faults and the length of time the organism has been subjected to their influence

The first effect of such unsatisfactory foods to which reference must be made is the *low standard of physical efficiency* which they induce both in man and his domestic animals In no country in the world is this more clearly manifested than in India *where malnutrition is so widespread and where food habits are so much* controlled by custom and by prejudice No one who has travelled far in India can have failed to notice the great differences in physique of different Indian races The poor physique the lack of vigour and of powers of endurance of certain southern and eastern races provide a remarkable contrast to the fine physique and hardiness of certain stalwart races of the north these differences are in the main attributable to differences in biological value of their national diets The low standard of physical efficiency *of man's domestic animals in certain parts of India is common* knowledge it has the same malnutritional basis and the gravity of its influence on the well being of the people can hardly be over estimated

In addition to lowering the standard of physical efficiency (a matter of vast economic importance to India) food which is faulty with respect either to suitable *protein to mineral elements to vitamins or to all three gives rise to many minor manifestations of ill health* without it may be the production of any morbid state to which we can attach a diagnostic label It inevitably leads to some deviation from the normal histological structure and to a corresponding reduction in functional efficiency of one or other of the various organs and tissues of the body the nervous the osseous the muscular the endocrine the gastro intestinal the respiratory and the circulatory systems It leads also to some deviation of the body fluids from their normal constitution the blood the lymph the digestive juices the secretions the excretions even the tears are all altered in one way or another each alteration contributing to or being indicative of impaired well being

It is to be recognized whether we be dealing with animals under experimental conditions or with man in his free state, that it is the gross evidence of malnutrition which force themselves upon our attention, which are recognized clinically and for which alone our system of nomenclature provides appropriate labels The lesser manifestations of malnutrition often escape our observation altogether although they 'affect the health of individuals to a degree most important to themselves' (Hopkins, 1906) If we closely observe animals subsisting on faulty food—even though the fault be not so great as to cause such wreckages of health as *scurvy, beri beri, pellagra, rickets* or *keratomalacia*—we notice many signs of impaired well being which have their counterpart in human subjects similarly situated with respect to the quality and balance of their food Thus, we may notice sub normal or, as I prefer to say, sub optimal states of growth or of unbalanced growth , or we may find that the animals' 'condition' is not so good as it might be that their coats lack lustre, or that they are dull eyed and devoid of the beauty of the well nourished animal , we may notice also that their excreta are not wholly normal, that they age prematurely, that their fertility is impaired, that they have but poor success in rearing their young, that their offspring when reared are very prone to disease and that the mortality amongst them is high We may find too, that they are apprehensive and timid, peevish or it may be ill natured, and that they resent handling which the well nourished animal never does all of which is unmistakable evidence of an unstable nervous system Yet such animals may be suffering from no nameable disease though they are obviously not well Similar symptoms of sub normal health are common enough in human beings , but since they may conform to no stereotyped disease, have no 'microbe' nor any toxin' associated with them, nor be accounted for by any laboratory tests which we apply to them we are apt to find nothing wrong with sufferers from them and to mistake their malnutritional meaning Obsessed by the idea of the microbe, the protozoa or the invisible virus as all important excitants of disease, subservient to laboratory methods of diagnosis, and hide bound by our system of nomenclature, we often forget the most fundamental of all rules for the physician, that the right kind of food is the most important single factor in the promotion of health and the wrong kind of food the most important single factor in the promotion of disease I emphasize these minor manifestations of malnutrition because they represent the beginnings of disease, and their recognition is, to my way of thinking, vastly more important than that of the wreckages of health, which even the man in the street can see, though his name for them may be less sonorous than our own

Next in importance to the *physical inefficiency* and the *minor manifestations of ill health* induced by faulty food come those gross states of morbidity that are due to specific food faults the list of these increases year by year I shall do no more than mention them, leaving you to link each to its own food fault They are —*keratomalacia, night blindness dental caries, polyneuritis, beri beri, pellagra, scurvy, rickets osteoporosis, slow healing of fractures, sterility, stone-in the bladder anæmias*, some types of *goitre, alimentary dystrophy, gastric atony and dilatation,*

diarrhœa constipation intestinal stasis colitis unhealthy skin disordered action of the adrenal glands and *vesical irritability* The results of animal experimentation have been generally accepted as demonstrating the ætiological relation of specific food faults to some of these maladies in man while their prevention in human beings by correction of the food faults has afforded incontrovertible evidence of the truth of this relationship But in regard to others the knowledge that they have a similar ætiology has been slow to diffuse and therefore slow to be put in practice though it has been arrived at by the same means Nevertheless it will ultimately be made clear in man himself that certain disorders of the gastro intestinal tract are as readily preventable by a perfectly constituted diet as are scurvy rickets or beri beri

The morbid states which are known to result from faulty nutrition in man s domestic animals are —imperfect growth slow development tendency for stock *to decrease in size deterioration* of imported stock and of their offspring high mortality low birth rate sterility reduced carrying capacity poor physique as draught animals low milk yield poor quantity of milk poor coats non parasitic skin diseases abnormal craving *for bones earth or fæces emaciation pernicious* anæmia one type of goitre cretinism hairless disease ' *lam ziekte* rickets *osteomalacia poor bone fragile bones swelling of joints stiffness of hind* quarters and lameness (*stijfziekte*) pining in sheep poor egg production in fowls and infertility of eggs These manifestations of malnutrition in man s domestic animals are attributed by veterinary scientists to deficiency in the food of essential mineral elements and with good reason But some of them such as low birth rate sterility rickets non parasitic skin diseases some types of goitre cretinism and pernicious anæmia can be produced by other means in laboratory animals deficiency of certain vitamins causing some and infectious agencies others It would seem therefore that a deficiency either of certain vitamins or of certain salts may bring about the same or apparently the same morbid result and that in regard to these and other essential constituents of food we have still much to learn as Hippocrates *puts it of the effects of each of them to every one* However this may be it is clear that the relation of malnutrition to disease production has many aspects and that progress in its comprehension depends on the closest co operation between students of human of animal and of plant nutrition

The mention of *lam ziekte* in cattle introduces us to a novel sequence of events in disease production This condition is due to a pathogenic agent—the *Parabotulinus bovis*— which has its habitat in decaying bones The primary cause of the disease is however a deficiency of phosphorous in the food of cattle which induces in them so great a craving for this element that to satisfy it they eat the bones in which the pathogenic agent resides thereby becoming infected It may be that in this observation there lies a general *principle which has an application* to mankind

This brings me to one of the most important means by which disease is brought about both *in man and animals by* faulty nutrition namely by increasing their

susceptibility to infectious agents During the past two and a half years 2 163 rats living in my laboratories under conditions of perfect hygiene have been fed on various faulty foods while the daily average of control or well fed stock rats was 865 The mortality in the ill fed animals (excluding those that were killed on the conclusion of certain experiments) was 31 4 per cent while in the well fed animals it was less than 1 per cent Of the former 13 5 per cent died of *lung diseases* chiefly pneumonia or broncho pneumonia while only 0 63 per cent of the latter died from the same cause Of the ill fed animals 3 3 per cent died of *acute gastro-intestinal disease* of the well fed only 0 09 per cent During the same period 1 252 previously healthy pigeons were fed on various diets deficient in vitamins the average number of stock or control well fed birds being 331 Of the ill fed birds 5 8 per cent died of *heart disease* chiefly pericarditis while of the well fed birds only 0 06 per cent died from this cause these figures being exclusive of 137 birds which developed true beri beri with its accompanying heart disease In the course of my own work I have seen true *dysentery* arise in ill fed monkeys while well fed monkeys living in the same animal room escaped and I have seen ill fed pigeons become infected with *Bacillus suipestifer* and with the invisible virus of *epithelioma contagiosum* while well fed birds living in their immediate vicinity escaped these infections The bacillus of *mouse typhoid* kills on injection over 90 per cent of ill fed mice while it kills less than 10 per cent of well fed mice the ill fed mice are likewise less resistant to *B pestis caviæ* and to *botulinus* toxins Birds are rendered susceptible to infection by *anthrax* when fed on food deficient in vitamin B and rats to *septic broncho pneumonia* when fed on food deficient in vitamin A guinea pigs when fed on food deficient in vitamin C die more readily from *tuberculosis* new born calves deprived of colostrum develop *interstitial nephritis* due to *B coli* infection swine suffer from *tuberculosis* which can be eradicated from the herds by well balanced vitamin rich food stock animals develop *sarcosporidia* from the same malnutritional cause Man himself provides many examples of a like kind I need but mention two In Northern Melanesia the native diet has been shown to be deficient in suitable protein mineral elements and vitamins and the poor physique of the natives and their high death rate from *respiratory* and *intestinal diseases* have been correlated with these deficiencies in the food, outbreaks of broncho pneumonia in children have been definitely traced to the inadequate ingestion of fat-soluble A and have been caused to disappear by the adequate provision of this vitamin This list of infectious diseases to which animals and man are rendered highly susceptible by faulty food is comprehensive enough including as it does respiratory disease gastro intestinal disease heart disease throat disease kidney disease and skin disease, and infections by such diverse organisms as protozoa bacilli and invisible viruses There is good reason therefore for the assumption that such death dealing diseases as tuberculosis, leprosy, cholera dysentery plague and malaria have often in this country a malnutritional element in their genesis and course Yet in attempting to combat them or to ascertain the conditions favourable to their spread how often do we

remember the prime rule of medicine that the wrong kind of food is the most important single factor in the promotion of disease? *I do not wish to detract* from the importance of bacterial or other living agents of disease, but I would say this that we know enough about their manner of life in test tubes and too little about their manner of life in the tissues of the body and the conditions therein which control their growth

Within recent years the spectacular results which have attended the experimental study of vitamins have overshadowed much else in nutrition both in the minds of the profession and the public' (Mendel 1923) It may not be inappropriate therefore to refer to a class of disease which results from the lack of balance of various components of the food, each component in itself good One example of the kind is afforded by the *hyperplastic goitre which may result from an* excess in the food of so homely a substance as butter The excess of butter or of unsaturated fatty acid causes thyroid hyperplasia by reason of the relative deficiency of iodine brought about by this excess similarly *enlargements of the thyroid gland of the colloid type* may be induced by an excess of lime they are preventible by increasing the iodine ingested proportionately to this excess Another example of much the same sort is that of *stone in the bladder* which is brought about in rats by ill balanced diets containing much oatmeal whole wheat flour or white flour To avoid stone the excess of these cereals must be compensated for by the consumption of appropriate amounts of milk Those most excellent foods oatmeal and whole wheat flour—the staple articles of diet of such vigorous races as the Scotch and the Sikhs—may likewise prove harmful by causing disturbance in the normal processes of calcification when *but only when* the diets containing them are poor in vitamin D These cereals are not in themselves complete foods a fact of which the races using them as staple articles of diet are not wholly in ignorance the Sikh does not attempt to subsist on *atta* (whole wheat flour) alone nor the Scot on oatmeal Any ill effect which these two foods may exercise is due to the failure suitably to combine them *with other food materials which compensate for their* defects They are not to be condemned nor to be displaced from their prominent place in the dietaries of mankind for this reason As well might we condemn the perfectly good fuel petrol for the over heating of the engines of our cars when we fail to supply them with sufficient oil as to condemn the excellent wheat and oats when we *fail to consume with them sufficient quantities* of milk or other vitamin rich foods which are required by the human machine for its smooth and efficient running The same kind of misunderstanding surrounds the controversy which periodically rages over the relative values of white bread and bread made from whole-wheat flour Both are excellent foods though neither is a complete food and since man requires a certain amount of suitable protein of mineral salts and of vitamins as well as of carbohydrates the superiority of the one bread over the other as the staple article of diet lies in the extent to which it excels as a source of these essentials Seeing therefore that white bread is notably more deficient in suitable protein in vitamins (both A and B) and in certain essential salts than

bread made from whole wheat flour or indeed than any other single food entering into the dietaries of western peoples (with the exception of sugars starches and fats which are marketed in the pure state) (McCollum) it is by this much the poorer foundation upon which to build a well balanced diet Those who can afford to build upon it and who possess the requisite knowledge to build wisely have little need to fear nutritional ailments though their building is improvident while those who cannot—and there are millions of such—are in grave danger of disease Next then in importance to the quality of the various ingredients of our food is their right combination

Of all the constituents of food on which normal health is dependent vitamins are the most remarkable We know neither what they are nor yet how much of them we need though knowing that normal metabolism is impossible without them We are accustomed to think of them in such infinitesimal terms that we have come to believe that the amounts we need of them are almost imponderable I do not know whether they are ponderable or not nor whether science will ultimately succeed in encompassing them all within chemical formulæ but I do know that for optimum well being we need much more of them than is generally supposed At all events races like the Sikhs whose physical development and vigour are equal to those of any race of mankind and superior to many consume these substances in large quantities as compared with races whose physique is poor I find that for rats the well balanced vitamin rich diet of the Sikhs is superior to any synthetic diet I can devise and to which vitamins in the form of yeast and cod liver oil are added I do not believe that human beings can have too much vitamins when they are taken in the form in which Nature provides them in well balanced combinations of unsophisticated food materials Some individuals appear to require more vitamins than others size being an important factor in determining their requirements some species of animals require more of a particular kind of vitamin than do others more are needed for reproduction than for growth and more for optimum well being than for the prevention of the named deficiency diseases more are required by the lactating than by the non lactating animal and more for longevity than for a shorter life The amount needed varies with the composition of the food with its balance in other essentials and with its digestibility more of one vitamin is required when the food is very rich in another as for instance more vitamin C when the food is rich in vitamin D there is for optimum nutrition an ordered balance even amongst the vitamins themselves In short the amount of vitamins needed varies with the metabolic requirements of the individual the attainment and maintenance of physical perfection heavy work reproduction lactation digestion exposure to cold infectious and debilitating diseases are all indications for their liberal supply

Before bringing this brief survey to an end I may perhaps refer to another aspect of the matter the effect of vitamin deficiency in increasing the susceptibility to certain poisons which the work of Smith McClosky and Hendrick has recently brought into prominence It has been mentioned that deficiency of vitamin A

increases the susceptibility of mice to *botulinus toxin* it also increases their susceptibility to mercuric chloride The same deficiency induces in rats an enormously increased susceptibility to morphine to ergotoxine and in lesser degree to histamine Deficiency of vitamin B likewise increases greatly the susceptibility of rats to ergotoxine and to pilocarpine Stimulants of the central nervous system are all more toxic to rats receiving too little vitamin A than to well fed animals Observations of this kind suggest forcibly that the ability of the tissues to detoxify certain poisons—both bacterial and other—is reduced by diets deficient in vitamins while indicating that such diets increase the sensitivity of the nervous system and of its autonomic division to toxic agents Not only may this be so but the disturbances of metabolism which result from vitamin insufficiency may themselves give rise to toxic metabolites which exercise specific effects on certain organs and tissues of the body This I believe to be the case in beri beri about which malady we shall presently engage in argument Most of us will probably agree that there is such a thing as a specific beri beri producing poison though disagreeing as to whether it be produced in rice before this food is ingested or in the intestine by some bacterial agent introduced with rice or in the course of a disordered metabolism arising out of vitamin insufficiency Our disagreements will not greatly matter so long as we recognize the prime importance of a sufficiency of the anti neuritic fraction of vitamin B in preventing beri beri

In looking through the pages I have just written I find mention of a host of diseases and of departures from health which make up an imposing array But amongst them there are none that I have not myself seen to arise as the direct or the indirect result of faulty nutrition or which are not vouched for by investigators of repute I know of no disease producing agency which reaps so rich a harvest of ill health as this though like others it has its limitations Perfectly constituted food is not a panacea for all diseases but it is an agent as potent in preventing a host of them as is the mosquito net in preventing one, or inoculation in preventing another while it is no mean co adjutor even to these

The newer knowledge of nutrition is I am convinced the greatest advance in medical science since the days of Lister and the sustained success of our profession in its conquest of disease depends in no small measure on the extended study of this vitally important subject and on the application in practice of the results reached by that study When physicians medical officers of health and the lay public learn to apply the principles which the newer knowledge of nutrition has to impart when they know what malnutrition means when they look upon it as they now look upon sepsis and learn to avoid the one as much as they now avoid the other then will this knowledge do for medicine what asepsis has done for surgery

DISCUSSION

Lieut Col A J H Russell I M S (Madras) Col McCarrison has in the course of his paper mentioned a large number of diseases and derangements of man and of animals which may be attributed in greater or less degree to defective nutrition and

faulty food No one, I think, will reject his thesis that each of these conditions may be linked up with its own food fault, nor do I think that any serious student of the subject would cavil at his statement that a tremendous amount of physical inefficiency and minor manifestations of ill health result from errors of diet Those of us who have spent any time in public health work in India are only too well aware of the physical inefficiency of great masses of the people of India, and this physical inefficiency undoubtedly lays them open to attacks of one or other of the great epidemic diseases

In the time at my disposal it is impossible —even though I were competent to do so—to discuss the relationship which all these malnutritional diseases have with defective food All that I would say is that in the past few years during which I have been responsible for initiating and carrying through preventive campaigns in Madras against the frequent cholera dysentery, and malaria epidemics from which the south Indian suffers so frequently and so intensely it has been borne in on me with ever increasing force that until we take up seriously the question of food nutrition and dietetics we will never get ' to the root of the matter

When one comes to realize the conditions under which the common people live, when one becomes acquainted with the actual food intake of the average ryot and his family one has only to apply one's knowledge of physiology to appreciate how little the ordinarily accepted and commonly practised preventive measures can do to retard development and spread of cholera and the other intestinal epidemic diseases There can indeed be little doubt that what are known as the intestinal diseases i e cholera, the dysenteries the diarrhœas colitis and gastro intestinal catarrhs, etc , are largely predisposed to by deficiencies of one kind or another in regard to food nutrition Why is the incidence of cholera in Madras 40 times greater than it is in the Punjab ? Why is it that in Madras city, according to a recent investigation gastro intestinal diseases are responsible for over 40 per cent of all deaths ? The reply is that Malnutrition is the means by which the soil of the human body is made ready for the rank growth of the pathogenic agents of diseases,' and once the soil is prepared other predisposing factors seize their chance We all know that carriers of cholera and dysentery exist in great numbers all over India and we all recognize the importance these carriers must have in the spread of diseases If, as Col McCarrison has shown dysentery can be produced under experimental conditions in animals merely by feeding them on food deficient in certain vitamins, is it too much too suggest that the human carriers of disease in India all the more readily develop and spread infection because of their defective nutritional condition ?

We have, in Madras, an extensive tract on the north east coast where beri beri is endemic Keratomalacia is also extremely common It has been proved that a high incidence of malaria and particularly endemic malaria is intimately associated with bad seasons, failure of crops, or, in other words defective nutrition Major Gen Hehir in his recent book on ' Malaria in India lays great stress on this factor In the Madras Presidency we have 300,000 deaths annually from fevers ' and a great proportion of these is almost certainly caused either primarily or secondarily, by infection with the malarial parasite

Recent expert opinion has stressed the importance of faulty nutrition as a predisposing cause of leprosy, another widely prevalent disease A low estimate of the number of lepers in Madras Presidency puts the figure at 120 000

Tuberculosis is without doubt spreading rapidly both in urban and rural populations in Madras The control of tuberculosis is not a problem by itself Rather is it a corollary to a host of other public health problems, one of the most important being nutrition and prevention being intimately concerned with the use of food of high biological value containing adequate amounts of vitamin A

Finally there can be no possible doubt that a large proportion of infantile deaths, and in some parts of Madras city, for example, the infant death rate is as high as 500 per 1 000 births have malnutrition as their root cause One has only to look at the mothers attending child welfare centres, dispensaries, and maternity hospitals to realize the truth of this proposition Malnutrition of infants is largely due, of course, to malnutrition of the mothers, but this merely shifts the burden to other shoulders, the original thesis remains unrefuted

All these, and other facts now available, point so definitely to the close relationship which *defective nutrition and food deficiency have* with disease and the subject is so intimately associated with food production that investigation of these matters is of the most supreme importance to all public health workers labouring for the advancement of the welfare of the people of India

I do not propose to urge the importance of the subject from the nutritional point of view, what I desire to impress on this audience is the necessity for the epidemiological study of these diseases leprosy cholera dysentery, tubercle, malaria and all the rest, if they are ever to be controlled The science of epidemiology has in the past, it seems to me received far too little attention in this country and, so far as I am aware, it has never been suggested that epidemiologists should concern themselves with malnutrition or food deficiencies Obviously, however, these factors must be taken into account in all epidemiological investigations, and in any enquiry into nutritional diseases which may be planned or conducted in this country, no team of workers will be complete, however many physiologists, pathologists and biochemists are included, unless a trained epidemiologist is given a place in that team Col McCarrison, I think has laid too little stress on this aspect of his research work, although this may have been an error of omission rather than one of commission May I conclude by giving an amended version of his concluding sentence ? When we know what malnutrition means then will this knowledge do for preventive 'medicine what asepsis has done for surgery'

NORMAL BASAL METABOLISM OF INDIANS

BY

MAJOR S S SOKHEY, I M S,
Haffkine Institute, Parel, Bombay

THE measurement of basal metabolism besides being of great value in the diagnosis and treatment of certain endocrine disturbances, has assumed great importance in the study of some of the physiologic phenomena underlying nutrition But for any work to be taken up in India there first must be standards of measurement Up to the present the standards available have been worked out from determinations made on Europeans and Americans, and very little is known about the differences in the metabolism of the different races or even about the effect of climate During the last few years figures for normal basal metabolism have been reported from China and Japan and they disclose no significant differences due to race But Du Bois reports a piece of work done in Brazil by A Ozorio de Almeida who studied the basal metabolism of 10 white men and an equal number of Negro labourers using a Tissot gasometer His subjects showed an average metabolism 24 per cent lower than the generally accepted standards for the United States In taking up metabolic work, I felt that there was need of a study of normal basal metabolism of Indians I am using Collin's chain compensated gasometer (a modification of the Tissot gasometer) and Henderson's modification of the Haldane gas apparatus Determinations are being made under conditions which have now become standard for such experiments, i e, the determinations are made 12 to 14 hours after the evening meal with the subject lying down in a state of complete repose So far I have studied 21 adult Indians between the ages of 19 and 30, almost all these subjects were medical men and it was easy to get co operation on their part I find that 15 of my subjects show a basal metabolic rate 10 to 23 per cent lower than the Du Bois standards I am not yet in a position to offer any explanation for this low basal metabolic rate but the work is proceeding

DISCUSSION

Dr H N Mukherjee (Bengal) From my experimental investigations with a Douglas bag—Haldane gas analysis apparatus on the basal metabolic rate of Bengalees (young students of the Carmichael Medical College) it appears that there is a definite lowering of the basal metabolic rate (about 9 per cent average) when compared to European standards This I attribute to deficient food These results were communicated to the *Calcutta Medical Journal* March 1926 and also in a note published in the *Journal of American Medical Association* in May 1926 Major Sokhey's findings are in agreement with my findings which go to support the theory of de Almeida

RELATIVE VALUES OF THE NATIONAL DIETS OF CERTAIN INDIAN RACES

BY

LIEUT COL R McCARRISON, C I E, M D, D SC, LL D, F R C P, I M S,
Director, Deficiency Diseases Inquiry, Indian Research Fund Association

THE relative values of diets, designed to resemble those used by the Sikhs, the Pathans the Mahrattas the Gurkhas, the Kanarese, the Bengalees and the Madrasees, were determined by biological assay on groups of rats of the same initial body-weight and age, the sexes being equally distributed in each group, and the animals being caged under precisely similar conditions of life To avoid sameness of food and its malconsequences, the menus were changed daily, the food being prepared with the same attention to culinary detail as for human beings The values of these diets were in the order in which they are named below, the Sikh diet being the best, the Madrasee diet the worst —

The Sikh diet was made up of whole wheat flour cakes (*chapattis*), milk, butter, dhal, tomatoes, abundance of root and green leafy vegetables, and fresh meat once a week

The Mahratta diet was made up of home pounded rice and whole wheat flour in equal parts, dhal, vegetables, milk, ghee, fish or eggs twice a week, tea and sugar

The Pathan diet was made up of cooked meat (daily), whole wheat flour cakes (*chapattis*), sheep fat, ghee, buttermilk, dried fruits, tea (twice a week) and condiments (salt, onion, garlic, turmeric, 'zeera,' cloves, darchine and a little red pepper)

The Gurkha diet was made up of rice, meat or fish or eggs, dhals, vegetables, milk, milk products, condiments (as in the Bengalee diet) tea and various 'puris' or 'paraunthas' (made of whole wheat flour fried in vegetable oil or ghee)

The Kanarese diet was made up of *ragi* (millet) rice once a week, a kind of soup made of tamarinds, onions and pepper with some vegetable and vegetable oil, and a little milk added to coffee

The Bengalee diet was made up of washed rice ('bhat'), and dhal 'puris' or 'paraunthas' (as in Gurkha diet), sweetmeats, fried gram, fish occasionally, milk and milk products (sparingly), vegetables (sparingly) and condiments [black pepper, red pepper, cloves, darchine, turmeric, onion, garlic (occasionally), asafœtida, coriander seeds and 'zeera']

The Madrasee diet was made up of washed polished rice, dhal (sparingly) and vegetables with condiments (kolumbu), 'itli,' 'dhosai' and 'vadai' (cakes of rice prepared with or without gingelly oil in various ways), coffee with sugar and a little milk buttermilk (occasionally), ghee (very sparingly), and betel nut and 'chunam' (lime)

The differences in nutritive value of these diets were marked The Sikh, the Pathan and Mahratta diets formed one group of high biological value, the Bengalee, the Kanarese and the Madrasee, a second group of low biological value, while the Gurkha diet occupied an intermediate position between these two The high value of the first group of diets was due to their high content of suitable protein, vitamins and mineral elements the low value of the second group to their low content of these food essentials The striking differences in the physique of different Indian races are due in the main to differences in biological value of their national diets

EFFECTS OF FAULTY FOOD DEFICIENT IN VITAMINS ON THE GASTRO INTESTINAL TRACT

BY

LIEUT COL R McCARRISON, *C I E , M D , D Sc LL D F R C P , I M S ,*
Director Deficiency Diseases Inquiry Indian Research Fund Association

THE health of the gastro intestinal tract is dependent on an adequate supply of vitamins of the A B and C classes These vitamins play an important part in absorption in the efficient functional activity of the digestive and neuro muscular mechanisms of the tract and in the freedom of the intestine from attack by bacterial or protozoal agents

The characteristic effects of deficiency of vitamin A in causing degenerative changes in the ocular tissues—the para ocular glands the conjunctiva and the cornea—and diminished lysozyme content of the tears with resultant invasion of the tissues of the eye by bacterial agents and the production of ophthalmia, keratomalacia and panophthalmitis are not confined to the tissues and fluids of the eye but may be observed also in those of the intestines where they give rise to patho logical changes of a like nature and to morbid states of a like kind Deficiency of this vitamin is specifically associated with ocular lesions mainly because the eye is so easy to see but when we make complete post mortem examinations we find that it gives rise to lesions of like kind in other membranes such as those lining the bronchi the bladder, the hilus of the kidney and the intestinal tract Vitamin A is to be especially associated with absorptive processes notably of fats in the small intestine

Vitamin B is now known to be of dual nature and to contain an anti neuritic as well as a growth promoting fraction It is not possible, in the present state of knowledge to separate the effects on the gastro intestinal tract of deficiency of the one fraction from those of the other but on general principles we may assume that the normal functional activity of the nerves and ganglionic plexuses controlling the movements of the gastro intestinal tract depends upon a sufficient supply of the anti neuritic fraction Thus in pigeons and fowls fed on polished rice (which is very deficient in this vitamin) the movements of the crop are greatly impaired This defect of motor function occurs also in other parts of the gastro intestinal tract Reduction or disappearance of gastric and intestinal contractions have been demonstrated in isolated portions of intestines by physiological methods and by X ray examination in the living animal Deficiency of the second fraction of

vitamin B (which is thought to be identical with Goldberger s anti pellagra vitamin) is the chief cause of the gastro intestinal lesions which characterize this disease In addition to the defects of motor function which result from deprivation of vitamin B there is said to be a reduction in digestive ferments—amylase lipase trypsin and rennet—as well as of ferments dealing with tissue oxidation Provision of the missing vitamin restores these ferments

Deficiency of vitamin C leads to intestinal hurry and to congestive hæmorrhagic and atrophic changes which may be widely distributed throughout the entire tract but are commonly more marked in the duodenum where actual ulceration has been observed by me in guinea pigs fed on a scorbutic diet of crushed oats and autoclaved milk

So much for what one might call the specific effects of deficiency of these three vitamins on the gastro intestinal tract but for my own part I prefer to attempt no differentiation of these effects but to group them all under the broad general heading of the effects of faulty food deficient in vitamins seeing that all three are essential to normal gastro intestinal function and that in nature their deficiency is practically always associated with other food faults which add their quota to the general morbid result

The effects of faulty food deficient in vitamins were first described by me in 1918 19 since that date confirmation of my observations has been provided by many other observers notably by Lumiere Cramer Findlay Gross Cowgill Plummer Deuel Messer Ohomori and his colleagues and Drummond I can do no better therefore than give here my original summary of them They are —

(1) Alimentary dystrophy
(2) Impairment of digestive and absorptive processes
(3) Congestive necrotic inflammatory (and sometimes ulcerative) changes in the mucous membrane of the tract sometimes involving its entirety sometimes limited to certain areas
(4) Degenerative changes in the neuro muscular mechanism of the tract tending to cause dilatation of the stomach ballooning of areas of the small and large bowel and probably also intussusception
(5) Degenerative changes in the secretory elements of the tract of the gastric glands the pyloric glands the glands of Brunner the glands of Lieberkuehn and the mucous glands of the colon
(6) Toxic absorption from the dystrophic bowel evidenced by swelling and discoloration of the mesenteric glands
(7) Pronounced reduction in the numbers of lymphoid cells throughout the tract with possibly inadequate amino acid absorption
(8) Impairment of the protective resources of the gastro intestinal mucosa against infecting agents (bacterial and protozoal) this impairment resulting not only in infection of the mucous membrane and under lying coats of the bowel but in the passage of micro organisms into the blood stream from the bowel

It is to be emphasized that these pathological changes are more marked in some individuals than in others, and that while all of them may occur in the same animal it is usual to find considerable variation in their incidence in different animals New growths in the stomach of monkeys and rats have also been occasionally observed to follow the long continued consumption of faulty food deficient in vitamins

The clinical manifestations of these pathological changes include gastric atony, diarrhœa, dysentery, constipation, chronic gastro intestinal cartarrh (mucus disease) colitis and intestinal stasis

DISCUSSION

R B Dr Chuni Lal Bose (Bengal) My first duty is to thank Col McCarrison for bringing this important subject for discussion before this august assembly It is of vital importance to the people of India, as national efficiency is indissolubly connected with the diet consumed by the people Col McCarrison has explained to us the difference in and the value of diet used by the people of the different provinces of India and how this affects the physique, health and activities of the respective races We all agree with him that the diet of the Bengalees is very poor in nutritional qualities It is a very ill balanced diet, it is too rich in carbohydrates, and sometimes in fat also but very deficient in protein elements The result is seen in the poor physique of the people, their disinclination to take to physical exercise and do active work, and the lowering of their general vitality and power of resistance to disease I shall give you a concrete instance of the evil effects of a diet poor in protein on the health of our students In a certain boarding house in a Government College, there were two sets of students, Anglo Indian and Indian, of nearly the same age living in a similar environment and performing the same kind of work intellectual and physical There was only one difference the quality of their food The Anglo Indians lived upon European diet and they got a daily allowance of three ounces of protein, whereas the Indian students lived upon an Indian diet yielding about two ounces of protein daily A record of the health conditions of all these students, kept for three years, showed that, while the Anglo Indian students showed an average increase in weight of 6 lbs, the Indians did not show an increase of more than 2 lbs Only 2 per cent of the Anglo Indian students lost weight during this period, while 42 5 per cent of Indian students lost weight The Anglo Indian students gained, on an average, an increase of two inches in chest girth, while the increase in the case of the Indian students was a mere trifle This experiment was carried out in the physiological laboratory of the Calcutta Medical College by Col McCay, I M S This shows how the Bengalee diet deficient in protein, prejudicially affects the physique, the health and efficiency of the student community of Bengal

If you compare the health and stamina of the people of east and west Bengal, you will find a marked difference The people of east Bengal are superior in physique and health to the people of west Bengal This is due to the difference in their diet The eastern Bengal people get plenty of fish and milk whereas these food stuffs (rich

both in proteins and vitamins) on account of their costly nature, are only available to the few in western Bengal in proper quantity

Mr J. T Edwards (United Provinces) Sir, I rise to criticize, for the most part adversely, certain of the statements made by Col McCarrison in the course of his opening address. I am prompted to do so for the reason that they may give rise to impressions concerning the nature and spread of certain diseases that are entirely erroneous Col McCarrison's efforts to propagate the study of nutrition are certainly meritorious, but when he enters lightly the realms of comparative pathology and contagious disease, I cannot help but think that he treads, for him, dangerous ground It is now well known as the outcome of recent research, that there occurs extensive derangement in the domesticated animals, osteoporosis, osteomalacia, and other conditions touched upon by the essayist, as the result of a deficiency in certain elements (calcium, phosphorus, or both and iron) in the food supplies available in the territories in which these diseases occur Further the losses caused by merely insufficient nutrition, particularly in India, are enormous

Nevertheless, when Col McCarrison gives vent to such statements as any morbid state to which we can attach a diagnostic label is connected, somehow or other, with malnutrition or defective metabolism, one cannot help but counsel him to exercise some caution He has given us a number of examples, mainly the outcome of laboratory experiments on small animals, to illustrate the supervention of bacterial disease in animals sustained on a faulty diet It is dangerous to generalize from such information Veterinary scientists can readily call to mind examples of naturally occurring diseases in which the origin of morbidity might, perhaps with equal reason, be associated with an opposite set of circumstances Thus the widespread infectious disease known as blackquarter in young cattle, prevalent also in India, has long been understood to attack, with particular severity in the scene of outbreaks, animals that appear to be in the best bodily condition, whereas animals that are suffering from debility are relatively resistant The well known infectious disease of sheep called 'braxy' is again said to attack the best conditioned animals in a flock The protozoan disease of cattle, known as tropical redwater, attacks full grown sturdy cattle with intense virulence, whereas very young and weak calves, which one might assume prima facie to possess very low power of resistance, react very mildly to infection The present very disturbing incidence of naturally occurring sterility among breeding cattle in Western countries can hardly, by any stretch of imagination, be laid to the charge of faulty nutrition, and the work done on the subject would incline one to believe that it is mainly associated with a chronic endometritis, the result of a bacterial infection, often secondary to the common specific contagious abortion

Then Col McCarrison recites a series of diseases tuberculosis, leprosy, dysentery, plague, which he holds have a malnutritional element in their genesis It is difficult to understand what he means by this statement, but if he wishes to convey that these diseases originate through any other agency than what we know, from a wealth of experimental research, to be the specific causal organisms of these diseases, nothing could be farther from the truth The enormous amount of work that has now been done upon tuberculosis in cattle proves conclusively that this disease is caused by the bacillus of tuberculosis, and can never occur except through the agency of this bacillus:

the only means of exterminating infection on an establishment where the disease exists is by getting rid of animals that carry the infection, and no amount of what is ordinarily termed good hygiene and improved nutrition can act as a substitute for this procedure Then we have in cattle a widespread important affection known as Johne's disease, which is very insidious in its development and which, in regard to its ætiological agent and the histo pathological changes it induces, bears considerable resemblance to human leprosy except that in the one disease the infection shows a predilection for the sub epithelial tissues of certain portions of the intestine, and in the other, of the skin Now Johne s disease arises and spreads rapidly among animals that inevitably wallow in vitamins notably on fertile pastures considerably overstocked with susceptible animals, when once the specific infectious agent has been introduced

The dissemination of specious explanations concerning the origin of such diseases would act specially disastrously upon the mind of the agriculturist in whose interests veterinary scientists are now endeavouring to instil exact notions based upon the available facts concerning the separate significance of faulty nutrition on the one hand and infection on the other

Col Eduard B Vedder (U S A) There is a great difference in infectious diseases Some, such as plague are so invasive and virulent that all infected contract them Others such as tuberculosis and leprosy are much more liable to be contracted by the ill fed In the case of leprosy many attempts have been made to infect healthy individuals by direct inoculation and with the exception of one dubious case in an Hawaiian criminal all have been unsuccessful It appears almost impossible to inoculate leprosy in a well nourished subject

Lieut Col R McCarrison I M S (B India) replied With the exception of Mr Edwards the speakers who have contributed to this discussion are in agreement with the thesis developed in my opening paper Mr Edwards has criticized my views adversely alarmed lest I be propagating a false gospel while adventuring lightly into fields unfamiliar to me I feel however, that he has misunderstood me or most probably that the continual opening and shutting of the door which interrupted the reading of my paper prevented him in a distant part of the hall, from hearing me aright Otherwise he would scarcely have attributed to me so exaggerated a statement as that ' any morbid state to which we can attach a diagnostic label is connected somehow or other with malnutrition or defective metabolism ' Nor could he have had any doubt in his mind that I wished to minimize the importance of the specific causal agents of such diseases as tuberculosis, leprosy, dysentery or plague My theme has been that malnutrition prepares the soil of the human body for invasion by pathogenic agents of disease a theme which can scarcely fail to contain a great measure of truth

A NOTE ON IODINE METABOLISM

BY

MAJOR CLIVE NEWCOMB D M, F I C, I M S

AND

GANAPATI SANKARAN, M B, B S

MONDA DEC 5 2 TO 4

EXPERIMENTS on the total iodine intake and output in human subjects are difficult and unsatisfactory on account of the difficulties in the estimation of minute quantities of iodine when mixed with an enormous excess of organic matter Many figures have been published of the iodine content of various food stuffs but in view of the difficulties we ourselves have encountered in this analysis we regard them, perhaps unjustly with some scepticism and especially those for rice which is the staple food stuff in South India We have examined several samples of rice and have failed to find any iodine in it It is not suggested that there is none but only that it is present in too small amount for estimation by the method we employed This method we think would have shown it if there had been as much as 2 parts in ten millions A diet of say 300 grammes of a rice containing only half this amount of iodine would however by itself supply 30 γ* of iodine a day and this amount, from others figures is probably enough for the needs of the human body

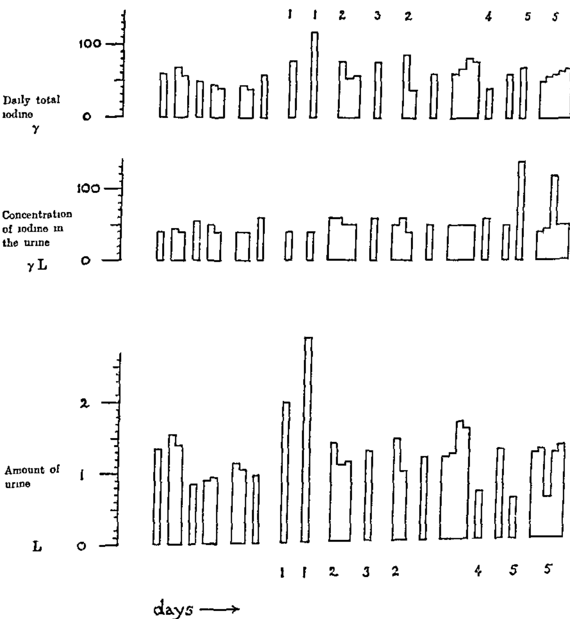
With regard to the output of iodine Fellenberg(1) and others have shown that it is mostly excreted in the urine and its estimation in this liquid is simple and more accurate than in food stuffs To test this conclusion to determine how the iodine excreted in the urine varied from day to day in a person on a constant and adequate diet and to find out what proportion of extra iodine given by the mouth could be recovered from the urine we have made the following experiments —

I A long series of controls and blanks were first done to determine with what accuracy we could estimate iodine in urine Fellenberg's method was used and the effect of various modifications in technique tried until a technique was arrived at which would consistently estimate to within 0 5 γ the amount of iodine in 50 c cs of urine, and give a recovery of added iodine within this figure

---

* γ = one thousandth of a milligram, i e 10^{-6} gramme

Diagram I



1 1 2 3 2 4 5 5
100
Daily total iodine γ
0
100
Concentration of iodine in the urine γL
0
2
Amount of urine
1
L 0
1 1 2 3 2 4 5 5
days ⟶

II For a subject a young Hindu student was taken living a quiet regular life in Madras on a constant diet which was presumably adequate in iodine His age was 27 and his body weight 47 kilograms His diet was —

Rice unpolished and parboiled	300 grammes
Dhall and other pulses	25 ,
Gingelly oil	5 c cs
Ghee	5 c cs
Condiments	
Vegetables	..
Skimmed milk	300 c cs

No tea or coffee and very little sugar

A record of the amount of urine passed was kept and the amount of iodine in 50 c cs of it estimated on 32 days between the 4th August and 21st November, 1927 The results are shown in Diagram I On most of these days the subject was living his normal life and eating a practically constant diet but on certain days indicated by numbers at the top of the diagram the effect of various changes of regime were tried

On the days marked 1 the subject drank as much water as he could get down throughout the day It will be noticed that although the quantity of urine was very much increased the amount of iodine in a litre of it was if diminished at all within the experimental error of the normal The total daily excretion was consequently much increased

On the days marked 2 the subject in addition to his usual diet took an extra amount of protein in the form of casein 25 grammes on the first of these two days and 50 grammes on the second This was taken late at night on the evening of the day before the iodine was estimated No demonstrable effect is produced

On the day marked 3 the subject took a strong purge also without demonstrable effect

On the day marked 4 the subject took his usual food but no water The quantity of urine fell but the amount of iodine in a litre of it was unaltered

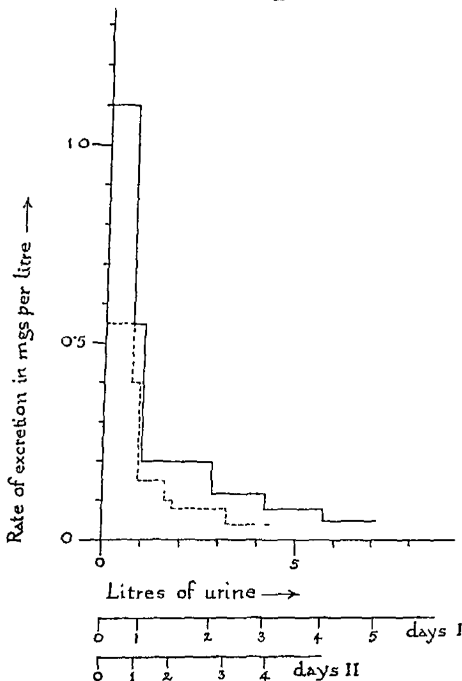
On the days marked 5 the subject took neither food nor water with the result of a very diminished output of urine and a very extraordinary increase in the amount of iodine in a litre of it bringing the daily total up to about normal

It will be noticed that for the most part the rate of excretion per litre of urine is remarkably constant and with the exception of the two days of total starvation always within the experimental error of 50 γ per litre The daily total excretion *per contra* is chiefly dependant on the amount of urine excreted It can be raised above its normal limits by increasing the amount of urine but the body appears to resist an attempt to reduce it much below normal on the two complete starvation days

III Two more experiments were done to determine what proportion of extra iodine added to the diet could be recovered in the urine and over what period it was excreted In the first the subject took 1 000 γ (1 mg) of iodine in the form of KI last thing at night and in the second 2 000 γ and the amount of iodine in the

urine was estimated on the subsequent days until it returned to normal The results are shown in Diagram II —

DIAGRAM II



It will be noticed that in each experiment about 40 per cent of the extra iodine (39 per cent in the first and 41 per cent in the second experiment) was got rid of on the first day after taking it. Taking the normal iodine excretion of this subject as 46 γ per litre of urine the total amount of extra iodine recovered comes to 555 γ (56 per cent) in the first experiment and 1 350 γ (68 per cent) in the second. In the second experiment the weather was cooler and the amount of urine excreted greater (average 1 118 c cs per day as against 1 160 c cs) and this may account for the better recovery.

We are indebted to the South Indian Branch of the British Medical Association for a grant for research from which the salary of one of us (Mr Ganapati Sankaran) was paid.

REFERENCE

(1) FELLENBERG TH VON (1926) Das Vorkommen der Kreislauf und der Stoffwechsel des Jods München

DISCUSSION

Lieut Col R McCarrison I M S (B India) Two points in Major Newcomb's paper excite my interest:—the relatively low iodine content of the diet eaten by the subject of his experiments and the fact that on this diet the concentration of iodine remains constant and varies directly with the amount of urine passed. If we are to believe all we read regarding the effect of iodine deficiency in causing goitre then the subject of Major Newcomb's experiments ought to have had goitre. I do not gather that he had and it seems not unlikely that his low intake of iodine was in balance with the general composition of his food. If Major Newcomb has found a diet which gives a constant excretion of iodine then he has overcome a great difficulty in determining the relation of iodine to other food constituents. Another point of interest suggests itself:—how the excretion of iodine is affected by changes in the thyroid gland such as occur in the various types of simple and exophthalmic goitre? It seems likely that work in this direction would yield interesting results. The relation of iodine to plant and animal life is a matter of very great importance and one about which we know little. The influence of iodine on the development growth and function of the thyroid is well known but this very knowledge appears to blind us to other matters of no less importance: its influence on growth in general both in animals and plants, its influence in increasing the absorption of nitrogen calcium and phosphorus: its effect on the yield and composition of milk: its potency as a catalytic agent—all these are matters about which we as yet know little. And if we know little about iodine in these metabolic relations how much less do we know about others of the mineral constituents of the food which exist therein in quantities almost as minute as the vitamins: manganese zinc nickel cobalt and radio active substances—all these call for investigation as urgently as does iodine. So while congratulating Major Newcomb on his interesting and important communication I would venture to express the hope that he and other chemists in India will devote attention also to the neglected mineral substances I have just mentioned.

Major C Newcomb I M S (Madras) replied The real difficulty in these experiments on iodine is the chemical one of estimating such minute traces of the element At present the chemical methods available are strained to the utmost Could a more delicate method be discovered we should, no doubt, be able to trace the effects of many of the changes in régime on the iodine output which are at present hidden in the experimental error It is hoped to continue the work and perhaps some day to extend it to other elements which enter in minute proportions into the animal metabolism.

A PRELIMINARY NOTE ON THE INTER-RELATIONSHIP OF SOME OF THE ENDOCRINE GLANDS IN SUGAR METABOLISM

BY

J P BOSE M B F C S (Lond)

Diabetes Research Worker School of Tropical Medicine and Hygiene Calcutta

THIS paper is based on a few interesting facts which came to my notice whilst working on the antagonistic action of insulin and adrenalin on rabbits I am presenting these facts to you in the hope that I may get help from those who have worked on similar lines or that others might be stimulated to work in the same direction I have not tried to draw any absolute conclusions from my results it is not possible to do so unless one has done a large number of experiments I have however thought fit to give a provisional opinion only in some instances

It will be remembered by many present at this gathering that when insulin came to India in the latter part of 1922 there was a good deal of controversy among the different workers as to its potency Some definitely asserted that insulin deteriorated in the tropics owing to the heat to which it was subjected when passing through the Red Sea especially in the summer I have had several samples of insulin sent to me by a well known London manufacturer in cold storage and my results in the standardization of these samples are in contradiction to those which Lieut Col Taylor of Rangoon found in 1924 i e that the samples of insulin did not differ much whether they were sent in cold storage or not It struck me then that it was not the heat in the tropics which was responsible for the so called deterioration of insulin and in collaboration with Col Acton we started to investigate the problem and to see if the individual variation in the rabbits had anything to do with this apparent loss or deterioration

It has been known for a long time that the various endocrine glands that stimulate the sympathetic nervous system play a large part in these individual variations and the first point we wanted to study was how far these variations in blood sugar by insulin depended on the individual variations in the rabbits before we attempted any assay methods We accordingly decided to test (1) the effect of the colour variations in these rabbits and (2) the antagonism exerted by adrenalin towards insulin

To find out the effect of colour variations in animals we selected for our experiments the albino the piebald and the jet black type of Himalayan rabbits and

also included the brown Belgian hare rabbit (which variety is generally used in the continent for insulin standardization tests) as a separate breed The results of our experiments the details of which have already been published were very striking The albino Himalayan type of rabbit was found to be most resistant to insulin while the brown Belgian hare type of rabbit was extremely susceptible to the same dose of insulin per kilo of body weight The jet black rabbit came next as regards susceptibility and the piebalds were even less susceptible than the jet black The most noteworthy feature of the result just mentioned was that the albino Himalayan rabbit is the most resistant and the brown Belgian hare type of rabbit is most susceptible to the same dose of insulin per kilo of body weight As a matter of fact the rabbits of the brown Belgian hare breed began to show hypoglycæmic reactions at the end of the first hour following the injections of insulin and many had to be revived by intravenous injections of glucose The jet black rabbits came next as regards susceptibility and 1 out of 13 showed severe hypoglycæmic reactions None of the white rabbits however developed the least sign of hypo glycæmia As a matter of fact they all behaved like normal animals throughout the experiments

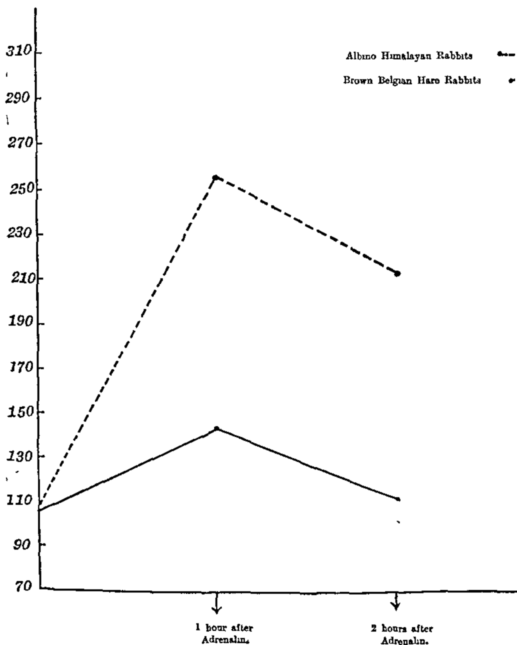
The question which naturally arose from these results was —What was the cause of this variability ? It is a known fact that the coloration of an animal is often closely associated with the function of the medullary substance of the supra renal glands and the literature contains a large amount of evidence to show that the action of adrenalin is directly antagonistic to that of insulin as it allows a larger amount of glucose to be available as blood sugar by converting the glycogen of the liver into sugar

In a previous experiment I found that small doses of adrenalin were capable of producing hyperglycæmia in rabbits and that when adequate doses of both adrenalin and insulin were given simultaneously, there was neither a rise nor a fall of blood sugar in rabbits Among other experiments which I did to show that adrenalin did cause hyperglycæmia I tested the effect of fright on the blood sugar of rabbits I found that when blood was drawn quietly without disturbing the animals the blood sugar findings were more or less normal but when the rabbits got excited by fright the blood sugar shot up to about 50 per cent higher than normal and on two occasions it became nearly double

Another interesting experiment which I did was to examine the urine of medical students before and after a *viva voce* examination and I found that 5 per cent of them showed glycosuria after such examination

These with plenty of other evidence in the literature show that adrenalin acts antagonistically to insulin though the action is not a direct neutralization of insulin by adrenalin but an indirect one Adrenalin acts through the splanchnic nerve and the hepatic plexus and causes a release of sugar from the glycogen store house of the liver and tends to cause hyperglycæmia Insulin at the same time causes the sugar to be taken up by the muscles which store it up as muscle and tissue glycogen tending to bring the blood sugar level down to normal

GRAPH.


310
290
270
250
230
210
190
170
150
130
110
90
70
Albino Himalayan Rabbits
Brown Belgian Hare Rabbits
1 hour after Adrenalin.
2 hours after Adrenalin.

Coming now to the question as to why the albino Himalayan rabbit should be so resistant to insulin and the brown Belgian hare so sensitive to it it seems reasonable to suggest on the basis of the evidence which I have just put forward that the *adrenalin content of the albino Himalayan* was high and as such it was capable of inhibiting the action of insulin to a large extent In the brown Belgian hare type of rabbit on the other hand it can be assumed that the adrenalin content was low and hence the insulin had a more or less unopposed action It may not be out of place to mention here that Col Acton and myself have found that human subjects suffering from leucoderma have a lower blood sugar content like the Europeans and respond less to insulin In conditions of hyperpigmentation on the other hand we have found that the patients react rather powerfully to *injections of insulin*

With a view to finding indirectly the adrenalin response of these rabbits of different breed and coloration the effect of injection of adrenalin on the blood sugar of these various types of rabbits was tested and it was found that in the albino Himalayan the blood sugar rises in an average from 0 11 per cent to 0 256 per cent one hour after the injection of adrenalin and falls to 0 224 per cent in the second hour In the brown Belgian hare type of rabbit on the other hand the blood sugar rises after the injection of adrenalin from an average initial level of 0 106 per cent to 0 145 per cent *only in the first hour* and falls very near to the normal level 0 114 per cent in the second hour

The above experiment clearly indicates that adrenalin acts very powerfully on the *albino rabbits liberating a large amount of glucose from the glycogen store* house of the liver which is capable of antagonizing the action of insulin In the brown Belgian hare rabbit the adrenalin response by the liver is very much less than that of the albino so that insulin is then capable of reducing the blood sugar to a much greater extent and producing symptoms of hypoglycæmia This experiment also brings home a rather valuable deduction viz that the albino Himalayan rabbits which reacted so poorly to insulin gave a well marked adrenalin response and vice versa the brown Belgian hare type of rabbit which *reacted violently* to insulin gave a poor adrenalin response These experiments also show that there is some association between the colour and species of rabbits and the output of adrenalin from the supra renal glands

We know *that in a healthy normal individual the fasting level of blood sugar* remains at a constant level It is usually increased after each meal and decreases after the effect of the meal has passed off The mechanism by which the amount of sugar in the blood is regulated and kept at a constant level is however very complicated We know that there are two groups of ductless glands with antagonistic actions which control the carbohydrate metabolism The first group consists of the pancreas the internal secretion of which helps the utilization of the sugar and thus it increases the carbohydrate tolerance The second group consists of the thyroid the supra renal and the pituitary the tendency of each of these three ductless glands being to mobilize the sugar into the blood and to diminish the sugar

tolerance The pancreas is influenced by the vagus and the secretions of the antagonistic group are all controlled by the sympathetic system

We have considered to some extent the mode of action of the supra renal glands, the adrenalin is believed to act through the liver by converting the liver glycogen into sugar and mobilizing it into the blood As has already been said, the secretion of adrenalin from the supra renal glands is under the control of the splanchnic nerves we also know that it is through these nerves that the impulses pass from the fourth ventricle and hence the puncture of the floor of the fourth ventricle causes hyperglycæmia and glycosuria

The role of thyroid in sugar metabolism is somewhat difficult to determine It has been proved that thyroxin, the active principle of the gland has no direct action on the blood sugar

TABLE I

Effect of insulin on the blood sugar of albino Himalayan rabbits

(Dose—3 units per kilo)

Serial number	Weight in grammes	Blood sugar before (percentage)	Blood sugar two hours after (percentage)	Percentage of reduction
1	1 390	0 10[illegible]	0 068	34·0
2	1 550	0 10[illegible]	0 068	31·0
3	1 800	0 11[illegible]	0 068	39 3
4	1 450			
5	1 100	0 086	0 060	30 [illegible]
6	1 370	0 07[illegible]	0 05[illegible]	[illegible]7 7
7	1 160	0 100	0 052	48
8	1 [illegible]00	0 095	0 056	41·0[illegible]
9	1 450	0 085	0·054	36 4
10	1 3[illegible]0			
11	1 590	0 107	0 053	50 6
12	1 370	0 123	0·06[illegible]	49 6
13	1 530	0 100	0 048	52·0
14	1 440	0 121	0 0[illegible]4	55 37
15	1 330	0 11[illegible]	0·0[illegible]6	3[illegible] 1

Minimum reduction 30 per cent.
Maximum 55 „
Average 48 „

The effect of thyroid feeding on animals has not given satisfactory results Krause and Cramer found that when small amounts of fresh thyroid gland are administered to rats or cats (fed on a carbohydrate rich diet) for 3 or 4 days glycogen disappeared from the liver On the other hand Burn and Marks fed rabbits for 18 days with thyroid (1 2 gr of fresh thyroid gland) and found that glycogen was still present in the liver But they said *that if thyroid feeding was continued long enough, the liver became free* from glycogen I am at present conducting a series of experiments in this line but the results hitherto obtained are not sufficient to warrant my giving any opinion as yet

To study the influence of the thyroid on the inter relationship of adrenalin and insulin which I have just described, I followed the following line of research I performed thyroidectomy on a series of 8 albino Himalayan rabbits which proved very resistant even to big doses of insulin and which also gave a marked adrenalin response I allowed a period of about a fortnight after the operation for the healing up of the wound and to get the animals back to a more or less normal state and then I repeated the experiments for testing the insulin response

The result of the insulin response on these thyroidectomized rabbits was *very striking (as will be evidenced from Table II when compared with Table I)* The very animals which were very resistant to insulin before thyroidectomy had severe reactions after the same dose of insulin and two of them died with typical hypoglycaemic convulsions I am also testing the adrenalin response of these rabbits and in 2 rabbits so far tested the adrenalin response is poor compared to what it was before thyroidectomy was done This work is still being continued

It seems to me that this is a valuable line of research because it tends to show that absence or deficency of thyroid secretion enhances the activity of insulin probably by altering the response of the liver to adrenalin stimulation Some observers have reported an increase in the islet tissues of the pancreas after thyroidectomy and it is believed that the thyroid has a direct inhibitory influence upon the pancreas

As regards the relation between the thyroid and the parathyroids it has been shown that they are antagonistic to each other the former stimulates the sympathetic and the latter inhibits the system The parathyroid is thus believed to be an ally of the pancreas in inhibiting the metabolism The evidence on this point is rather vague as yet

The thyroid secretion is a direct stimulant to chromatophil tissues causing them to yield adrenalin to blood in larger quantities this is the reason why the release of sugar from the glycogen store house of the *liver by adrenalin stimulation* is affected after thyroidectomy Thyroid thus takes a prominent part in the regulation of blood sugar

TABLE II.

Effect of insulin on the blood sugar of the same albino Himalayan rabbits after Thyroidectomy

Serial number	Weight in grammes	Blood sugar before (percentage)	Blood sugar two hours after (percentage)	Percentage of reduction	REMARKS
1	1,390	0 100	0 033	67	Had severe hypoglycæmic convulsions Glucose in injection given
2	1,550	0 104	0 036	65	Slight convulsions
3	1,800	0 110	0 027	75	Severe convulsions Retraction of the head and extension of the hind limbs Glucose given but died
5	1,100	0 088	0 037	58	
6	1,370	0 075	0 032	57	
8	1,200	0 098	0 021	78	Very severe convulsions Died in spite of glucose
9	1,450	0 088	0 035	60 2	Tremor, restlessness, rapid breathing
15	1,330	0 114	0 042	63 2	Severe convulsions, relieved with intravenous glucose

I have not yet worked out the relation between the pituitary and the pancreas, but from the literature one finds that the pituitary gland also helps a good deal in keeping the blood sugar at a constant level Injection of pituitrin causes an increase in blood sugar and can relieve the hypoglycæmia caused by insulin, though this is not so marked as by injection of adrenalin Sub pituitarism is known to lead to an increased sugar tolerance

The nature of relationship between thyroid gland and the pituitary body is at present somewhat obscure It has been found that hypertrophy of the pituitary body takes place after thyroidectomy sometimes to three times the normal size; but even then there are no symptoms of hyperpituitarism Hypopituitarism is also said to cause hypertrophy of the thyroid.

In addition to these experiments on rabbits, I am carrying on a series of experiments on this antagonism problem on human subjects I have selected two groups of cases for experiment those with loss of pigmentation, such as leucoderma, and those with increased pigmentation, such as chloasma, kala azar, etc Both these groups of cases, curiously enough, show a blood sugar on the low side of normal the reason of which I am not yet in a position to explain fully Anyway, the most remarkable feature of these groups of cases is that they show opposite results regarding their adrenalin and insulin response The leucoderma cases behave more or less like the albino Himalayan types of rabbits and the other group of cases behave like the pigmented rabbits The leucoderma cases show a high adrenalin response and a weak insulin response The kala azar cases, on the other hand, show a weaker adrenalin response but a more pronounced insulin response In fact, a few of the kala azar cases had slight hypoglycæmia reactions after insulin which were entirely absent in all of the leucoderma cases

EFFECT OF MANGANESE ON GROWTH.

BY

LIEUT COL R McCARRISON, C I E, M D, D SC, LL D, F R C P, I M S,
Director, Deficiency Diseases Inquiry, Indian Research Fund Association

MANGANESE is present in all living tissues—both vegetable and animal It is most abundant in those parts of plants in which biochemical processes are most active in young leaves and shoots and in seeds, wherein its presence appears to be necessary to the development of the young plant It is significant that manganese occurs most abundantly in those parts of plants in which vitamins are also most abundant Thus cabbage leaves contain as much as 0 64 mg per 100 grms of the fresh material, turnip leaves, 0 21 mg, asparagus tops, 0 10 mg, cress, 0 16 mg, leek, garlic and onion from 0 05 to 0 09 mg, while fruits—orange, lemon, strawberry, grape—contain in ascending order from 0 1 to 0 73 mg of manganese per 100 grms of the fresh material Whole wheat is particularly rich in manganese, while paddy is poor in it It appears to be concentrated in the outer layers of the wheat grain, Bertrand having found as much as 3 9 mg in each 100 grms of dry wheat bran, while estimations made for me by Dr R V Norris have shown the whole grain to contain 4 82 mg per 100 grms of the dry material Like vitamin B it is, therefore, largely removed in the process of manufacture of white flour

In animal tissues it is also present in largest amount in the organs of principal functional importance, the liver contains 0 170 mg per 100 grm of fresh tissue, the pancreas, 0 076 mg, the lymph nodes, 0 063 mg, the kidney, 0 061 mg, while the muscles, heart, brain, lungs, stomach, intestine and spleen, contain considerably less from 0 014 to 0 033 mg, in ascending order from muscles, which contains least, to the spleen, which contains most Manganese is also present in milk and eggs and is said also to occur in insulin It is present in the blood in fairly constant concentration in health 0 0001 per cent The administration of manganese dioxide by the mouth rarely does more than double this concentration, which soon returns to the normal level, the excess being arrested in the liver and excreted as soluble carbonate in the bile

Manganese is thus a substance of great physiological importance in plant and animal life It appears to act as a catalytic agent regulating the capacity of the tissue cells to utilize oxygen Its high content in the liver, pancreas and lymph nodes suggests an important relation to assimilative processes, a relation which

observations by Camescasse on debilitated children would appear to confirm In suitable dosage it augments the production of anti toxins, and accelerates the growth of plants, and—as the experiments here recorded show—of animals Given in large dosage either orally or subcutaneously to animals under experimental conditions, it produces cirrhotic changes in the liver and kidneys, while in the human subject chronic manganese poisoning gives rise to symptoms resembling those associated with progressive lenticular degeneration

In 1924 Levine and Sohn reported the results of certain experiments dealing with the effects of manganese on the growth of young rats They used manganese sulphate giving it in various concentrations ranging from one part in 2,000 to one part in 10 000 Their results indicated that manganese had 'a marked catalytic effect on growth The manganese rats proved more active than the controls, their coats of hair were sleeker, longer and thicker' But apart from this statement no particulars were given by them as to the comparative rates of growth in animals receiving manganese and in those not receiving it, nor as to the concentration of manganese in the food most favourable to growth

Experiments which I have carried out to determine the effect of different counteractions of manganese on growth of rats fed on an otherwise complete diet have yielded the following results —

1 Manganese dioxide when ingested by young rats to the extent of 0 889 mg daily—representing in terms of Mn, a concentration of one part of manganese to 12 600 parts of the food eaten—exercised a progressively increasing and retarding influence on growth This retarding action was manifested only after 32 days' consumption of the salt Its effect would appear to have been cumulative

2 Manganese chloride when ingested by young rats to the extent of 0 0327 mg daily—representing in terms of Mn, a concentration of one part of manganese in 617,700 parts of the food eaten—exercised markedly favourable influence on growth

The conclusion appears to be justified that concentrations of manganese of the former order are harmful to the animal organism, while concentrations of the latter order are beneficial And since a diet containing a fair proportion of whole wheat provides a concentration of manganese of the latter order, it may be concluded that the growth promoting properties of whole wheat are in part due to the content of manganese in this cereal

THE EXPERIMENTAL PRODUCTION AND PREVENTION OF STONE-IN-THE-BLADDER IN RATS.

BY

Lieut Col R McCARRISON, C I E, M D, D Sc, LL D, F R C P, I M S,
Director, Deficiency Diseases Inquiry, Indian Research Fund Association

The diets employed by me in the experimental production and prevention of stone in the bladder in rats have been composed of food materials in common use by people resident in the stone areas of India They are given in the following table —

	Percentage composition of the diets											
Diet Number	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	X[cut off]
Whole wheat flour		53		53	78	90	90		53	53		
White flour (American)								100			53	
Scotch oatmeal	53		53									
Cornflour (tinned)	25	25	25	25					24	25	25	
Linseed meal	20	20	20	20	20				20	20	20	
Sesame oil (gingelly)						8						
Olive oil (pure lucca)												
Linseed oil							8					
Milk (in ounces)			1	½								
Calcium phosphate	1	1	1	1	1	1	1		1	½	1	
Sodium chloride	1	1	1	1	1	1	1		1	1	1	
Potassium oxalate									1	½	.	
Salt mixture (McCollum)												
Water ..	q.s	q s	q s.	q.s	q s	q s	q s	q s.	q.s	q.s.	q s.	q

Of these diets 10 (I, II, V, VI, VII, VIII, IX, X, XI and XII) caused stone in the bladder and two (III and IV) did not Those that did not, contained milk to the amount of two thirds of an ounce per rat daily It follows, therefore, that the disease is due to the absence from the stone producing diets or to the presence in *insufficient quantity, of substances contained in milk* These substances are not present in vegetable oils in amounts sufficient for the prevention of stone They are fat soluble vitamins, in all probability of the vitamin A and not of the vitamin D class

The incidence of stone in the bladder varies with different diets and even with the same diet in different experiments It may range from 11 to 50 per cent of the animals fed on the faulty diets *An exclusive diet of American white flour* may also cause stone in the bladder but with less certainty than some of the other diets since the animals rarely live long enough to acquire the disease The malady is not due, therefore, to any 'toxamin' present in whole wheat or oatmeal but is a deficiency disease There is no very marked difference in the incidence of stone in the two sexes The stones are largely made up of phosphates of calcium and magnesium with traces of oxalate, uric acid being absent, but quantitative analyses are not yet completed The sequelæ of stone—dilated ureters, hydronephrosis pyelitis and pyonephrosis—are common Cystitis is not a necessary precursor of stone in the bladder in rats since a fair number of cases have occurred in which no macroscopic evidence of cystitis was present The inflammation of the bladder which accompanies the disease is very variable in its intensity sometimes it is great and of hæmorrhagic nature, sometimes it is slight, sometimes it is generalized and sometimes it is localized, inflammatory thickening around the neck of the bladder or at the orifices of the ureter or both are frequent, often so marked as to suggest new growth Sometimes cystitis is present without stone Stone in the kidney or in the ureter is an occasional accompaniment of stone in the bladder

Very little is known of the chemical composition of vesical calculus in man, partly because of the unwillingness of surgeons 'to have the trophies of their surgical operations broken up for chemical analysis,' and partly because of the erroneous notion that its chemical nature can be judged from its physical appearance (Moore 1911) A great variety of vesical calculi is described—uric acid or urate phosphate, oxalate, carbonate cystine, *xanthine fibrin, cholesterol, etc* But according to Benjamin Moore (1911), Iwano (1923) Praetorius (1925), Shea (1925), the phosphate and oxalate *of lime form the main constituents of calculi,* whilst uric acid and urates are only of third rate importance

DISCUSSION

Dr V G Heiser (U S A) It has been a pleasure to listen to these further contributions of Col McCarrison who has already done so much to show the relationship between *nutrition and disease In connection* with manganese I should like to ask Col McCarrison whether he has any observations as to the quantity of manganese in

cereals grown in different soils whether there is any difference for instance, in the amount of manganese in naturally watered and irrigated soils or in regions of different degrees of manganese etc

R. B Dr Chuni Lal Bose (Bengal) Students of chemistry present in this hall will remember that the first experiment they had to do in the practical class was the preparation of oxygen by heating chlorate of potash mixed with manganese dioxide This action was at one time believed to act as a catalytic action but now manganese is found to act as a carrier of oxygen We are told by Col McCarrison that manganese acts in the same way in the animal system in the process of nutrition It helps imbibition of oxygen by the tissues This is very interesting information and we are very thankful to Col McCarrison for carrying out so many interesting experiments to solve the complex problem of nutrition

With reference to his second paper I would enquire of Col McCarrison as to the chemical composition of the stones he found in rats fed on special kinds of food whether they were purely phosphatic or a mixture of phosphate and oxalate and if there was any trace of uric acid or urates in them It would help us to throw some light as to the relation between the chemical composition of the special kind of food and the stones resulting from the use of such food

Dr T S Tirumurti (Madras) Col McCarrison has given conclusive experimental proof that stones in the urinary bladder ureter etc in rats are produced by diets deficient in vitamin A But he has not stated how the stones are actually produced He is of opinion that cystitis and other inflammatory conditions of the urinary passages are the sequelæ of the formation of stones and are not antecedent conditions Our present conception of stone formation is that inflammatory conditions of the urinary passages lead to inflammatory exudates which upset the colloidal equilibrium of the urine in which the salts are dissolved leading to a precipitation of the salts Whether any such change in the colloidal equilibrium of the urine results from deficient diets is a subject on which I hope Col McCarrison will enlighten us The exact method of stone formation is yet to be explained The mere observation that deficient diets produce stones does not explain the whole pathogenesis of the disease

Lieut Col E B Vedder (U S A) A deficiency of vitamin A leads to degeneration of epithelium May it not be that such epithelial degeneration leads to a bacterial invasion of the bladder or kidney and that these bacteria serve as an excitant and nucleus of the stone ?

Dr H C Menkel (Punjab) Were any biliary stones observed as produced by the dietary restrictions ?

Dr P Sharma (Dhar State B India) Col McCarrison pointed out from his experiments in animals that diets of wheat etc are found to be associated with the production of stone in the bladder *My observation is that the Bhils in the southern* part of Central India suffer very frequently (at all ages) from this disease and their staple diet is maize (Indian corn) They eat very little wheat and rice The kind of stone mostly found is phosphatic There must be some connection between the formation of the stone and the diet of maize

Capt P Ganguli (Bengal) My own observations on the composition of stones and gravel in Bengal is that about 80 per cent are made of oxalates and phosphates

as determined by chemical analysis On analysing nearly a hundred calculi, I was surprised to find that only 20 per cent contained uric acid amongst other constituents If cats were fed exclusively on rice, oxaluria appeared within a fortnight and on post mortem examination, duodenitis was found in every case Oxaluria is exceedingly common amongst Bengalees These facts lead me to suggest that the faulty dietetics of the Bengalees, whose staple diet is carbohydrate in the shape of rice, which is deficient in protein, have got something to do with the formation of stones composed chiefly of oxalates

Dr Bhagwandas J Devidasani (Sind, B India) Col McCarrison said that stone is most frequent in Sindees and that milk is a good preventive of stone production As a Sindee I can say that Sindees (even of the poorest classes) take larger quantities of milk and its preparations, than most other races There must, therefore, be some other constituent in the diets or some other condition in the lives of the Sindhees which, in spite of an abundance of milk and its preparations, causes stone in Sind

Lieut Col R McCarrison I M S (B India) replied The estimation of the amount of manganese in different food stuffs and in cereal grains grown under different manurial conditions is at present being carried on in my laboratories It is hoped that a report of the results reached may be published during the course of next year

In reply to R B Dr Chuni Lal Bose the calculi produced in rats, under the experimental conditions I have indicated, are composed, for the most part, of phosphates, a sufficient number of analyses have, however, not yet been made to enable me to give Dr Bose definite figures of their composition

Dr Sharma draws attention to the association of a maize diet with vesical calculus in Bhils I have no doubt that he is correct in assuming that there is some connection between this diet and the production of stone My experimental observations are still at an early stage and much work remains to be done before the precise relationship of faulty nutrition to calculus production can be decided

DEFICIENCY DISEASES.

EPIDEMIC DROPSY ITS BEARING ON THE BERI-BERI PROBLEM

BY

LIEUT COL J W D MEGAW, C I E I M S,
Director, Calcutta School of Tropical Medicine

Tuesday Dec 6 10 A.M 1 P.M

To many of you who come from the further East epidemic dropsy is a mere name, those who have read of it in some of our standard textbooks may be forgiven for regarding it as a variant of famine œdema, most of you will think of it as a disease which is only of interest to Calcutta and a few other places in Bengal and you may feel somewhat impatient at being compelled to hear about it

In most of the affairs of life it is a sound rule to mind your own business but in tropical medicine we often find it necessary to keep an eye on the diseases of other countries as we never know when they may affect ourselves In the East we have no yellow fever, yet we keep a watchful eye on the disease as we have no assurance that it may not come home to us very closely at some future time

There are two reasons why epidemic dropsy should be of interest to all medical men in the East (1) the disease may not be so restricted in its distribution as we imagine, (2) it is probably one of the members of the beri beri family at any rate it is so closely related to beri beri that no student of that disease can afford to ignore it A study of epidemic dropsy may throw much light on the problem of beri beri For these reasons it is desirable that all of you should take an interest in epidemic dropsy and I will try to state a few of the outstanding facts and problems in connection with the disease It is only by stating these facts that I can tell you what the disease is, it is not possible to give you a crisp and clean cut definition of epidemic dropsy, this cannot be done until the cause of the disease has been demonstrated to the satisfaction of the scientific world

Epidemic dropsy is a name which was first employed by the late Col Kenneth MacLeod, I M S, of Calcutta as a designation for a disease which occurred in Calcutta in the winters of 1877 78, 1878 79 and 1879 80, disappearing in the hot weather after each winter outbreak Large numbers of rice-eating people in certain quarters and groups of houses in Calcutta were attacked and soon after the appearance of the disease in Calcutta it broke out among coolies in Assam and Mauritius As these coolies had come from Calcutta it was naturally assumed that the disease was communicable and that it had been brought from Calcutta by the coolies I

suggest that it was equally possible that the disease was caused by eating rice and that supplies of the affected rice were sent from Calcutta to Assam and Mauritius to feed the coolies This suggestion cannot well be tested after so many years have passed but it seems reasonable to assume that the same trains and ships which carried the coolies from Calcutta would also be used to convey supplies of their food stuffs especially as the coolies were going to places where local supplies of their customary food were not likely to be available Since the outbreaks described by MacLeod there have been many small explosions of the disease, chiefly in Calcutta and Howrah but also in other parts of Bengal and even in distant parts of the United Provinces and elsewhere in India

Two great outbreaks occurred in the years 1909 and 1926 in Calcutta the former was closely studied by Col F D W Greig I M S, who formed the opinion that it was due to a dietary deficiency and that it was a form of beri beri I had personal experience of the 1909 outbreak, having 13 cases of the disease under my care in the General Hospital The patients were Anglo Indians and poor Europeans into whose dietary rice had entered to a considerable extent All had swelling of the feet and legs ten had dyspnœa, five had palpitation, four had slight fever, seven had complete loss of the knee jerks, in the others the jerks varied in a surprising manner being exaggerated in some and in others being variable sometimes present and sometimes absent All had tenderness of the calves and two had dimness of vision associated with high intra ocular tension All of them *improved rapidly when placed on a nutritious diet free from rice From an early* typical case 3 c cs of blood was drawn and injected subcutaneously into myself without any obvious consequences I formed the opinions (1) that there was no justification for separating the disease from beri beri (2) that there was no evidence of person to person communicability (3) that the most reasonable explanation of the disease lay in a poison which developed in the rice during the hot and rainy season by a micro organism, and (4) that the secret of prevention lay in the careful storage of rice

These views were essentially the same as those held by Braddon in the case of beri beri but Braddon regarded epidemic dropsy as a distinct disease because he held that the poison which caused beri beri could only form on over milled rice whereas epidemic dropsy occurred in eaters of parboiled rice

Col Neil Campbell, I M S, in the *Indian Medical Gazette* of September 1908 had already suggested a poison formed in rice as the probable cause of a remarkable explosive outbreak of epidemic dropsy which occurred in the Dacca Lunatic Asylum in March 1908 *The total number of cases was 155 among a population of 270* and no less than 147 of the patients were attacked within a period of five days The diet was varied and wholesome and the persons who were attacked were mostly those who had previously been in good health rather than those who had been in bad health It is significant *that the rice in use at the asylum was bought in* quantities enough for two or three days' use I would suggest that one of the consignments of rice was responsible for the explosive outbreak

Since 1909 there have been numerous small outbreaks in Calcutta and Howrah and also in other places in Bengal Most of these have been explosive and have attacked strictly limited groups of people The outbreak of 1926 was the largest on record but it did not differ in any notable respect from the preceding ones It was really made up of a large number of explosions occurring over a large population in rapid succession in such a way as to produce a kind of thunder storm rather than an explosion

What is Epidemic Dropsy?

Epidemic dropsy is a name employed to designate a disease with certain features among which the following are conspicuous —

(1) It affects rice eaters There is no clear evidence of its occurrence among people who do not eat rice There have been a few reports of doubtful single cases in persons who had not eaten rice but these are not well authenticated and their weight as evidence is very small when we take into account the mass of opposing evidence to the effect that thousands of cases have occurred in rice eaters while those who lived in the same localities but did not eat rice invariably escaped The degree of incidence of the disease in an affected place corresponds in a remarkable manner with the extent to which rice forms a part of the diet When rice is the staple article of diet the incidence is high when a little rice is eaten the incidence is low, when no rice is eaten the incidence is nil

(2) The victims have nearly all been eaters of parboiled rice which has been stored for several weeks or months in a hot and damp place after manufacture The millions of people in rural Bengal who store their rice in the form of *paddy* and prepare only small quantities at a time are remarkably free from the disease Two small outbreaks have been reported in families which have stored their own *paddy* so that it is possible though exceedingly rare for the disease to occur under these conditions In these outbreaks the *paddy* had been stored in a damp condition

(3) In many of the outbreaks it has been possible to discover that all the affected persons have eaten rice which came from the same store although the households affected have been widely separated from each other

(4) Many of the smaller outbreaks have been explosive all the patients showing their first symptoms within a few days of each other even when they lived at considerable distances apart

(5) The disease does not run a course like that of an infection and if rice is cut out of the diet all early cases cease to progress it is only advanced cases in which serious damage has already been done to the tissues that fail to respond to a change of diet Some private practitioners have reported cases in which the disease has progressed although rice had been cut out of the diet Such cases have never been seen when the patients were under control in a well regulated hospital and it is likely that the exceptions which have been reported are apparent rather than real and are due to the patients reluctance to deprive themselves of their accustomed article of diet They may profess to have obeyed their doctor's instructions while they have continued to eat rice all the same

(6) The seasonal distribution is remarkably uniform in Calcutta it corresponds closely with that of beri beri in Japan *being usually in the rainy season* and after the rains When the new rice crop comes into use the disease disappears promptly

Outbreaks may occur at any season but they always affect persons who have eaten rice which has been stored under hot and damp conditions *Strangely enough* there have been rather striking outbreaks in the cold seasons following on the great outbreaks in Calcutta e g in Allahabad in the winter of 1926 27

(7) Infants escape cases have been recorded with increasing frequency in children from the ages of three till ten years Males and *females* are almost equally affected but the disease is more severe on the whole in females

(8) There is no evidence that the diets of the affected persons have been defective in any obvious respect when compared with the diets of the unaffected persons in the same localities In many cases the diets of the affected persons have been much more satisfactory in all nutritive elements including vitamins than the average diets of their neighbours

(9) The occurrence of the disease is not associated in any way with an obvious change in the diet The food seems to be just the same as usual but this circumstance does not exclude a food poisoning In the great majority of cases of food poisoning no obvious change has occurred in the food An article of diet which causes deaths from botulism may appear to be the same as a healthy one

Certain changes have been found in the rice which was in use by the victims of epidemic dropsy certain bacteria and poisons have been isolated by Col Acton and Col Chopra I am not competent to express an opinion on these points as they are outside of my province

(10) When several people in the same house or locality are attacked in rapid succession the most obvious explanation is that the disease spreads by infection Closer investigation however shows that many of the outbreaks can only be explained as being due to the simultaneous action of a morbid agency common to all the cases a few outbreaks have behaved in such a way that they could be explained either as being due to a pre formed food poison or to infection but in no outbreak has a person to person communication been the only possible explanation

Col Greig made a very careful study of this point and all my personal observations fit in *exactly* with his *conclusions that the disease is not infectious,* but is associated with the diet

(11) Most of the outbreaks can best be explained as being due to a toxic agent which is already present in the rice before it is eaten Some cases behave in *such a way that* we cannot exclude the possibility of a survival after cooking of the microbic agency which produces the poison and of the continued production of poison in the alimentary canal

This toxi infection view held by Noel Bernard in the case of beri beri would explain some of the experiences which have been reported in epidemic dropsy such

as the recurrence of symptoms in persons who had ceased to eat rice and then resumed the use of irreproachable rice The vast majority of the cases can best be explained as due to a pre formed toxin but toxi infection cannot be excluded as a possibility in some cases

(12) The disease has never been known to spread from patients who have gone for a change to places in which the disease is not liable to appear spontaneously Hundreds of persons suffering from the disease have gone to various places in Bihar and the United Provinces and have lived in close association with the people of these places and yet there is no record of the disease having been communicated to the people with whom they have associated The very rare cases in which the people of a place have been affected after contact with persons suffering from the disease have invariably occurred in affected localities never in non endemic areas

Some of the Chief Clinical Features of the Disease

(1) Although there is an infinite variability in the severity of the disease yet there are few maladies which are so uniform in their broad general characteristics or so easy of diagnosis The occurrence within a brief period of an otherwise unexplained dropsy in several members of a family or community, occurs only in epidemic dropsy or beri beri In famine œdema the diet is an obvious explanation, while in epidemic dropsy there is rarely any evidence of lack of nutritive elements in the diet

Very rarely there are sporadic slight cases of œdema in connection with which a difficulty of diagnosis may arise these are so exceptional that they are of little importance and their practical management causes very little trouble to the medical man

(2) With the swelling of the feet and legs or of the whole body there is nearly always shortness of breath palpitation on slight exertion and weakness Some degree of cardio vascular disorder is probably invariable but in very mild attacks this may not cause obvious manifestations except for the swelling of the feet

(3) There is often a slight degree of fever especially at the onset, this may persist for long periods of time in severe cases

(4) At the onset there is frequently an obvious gastro intestinal irritation in the form of diarrhœa with nausea or vomiting, there may also be dysentery

In severe cases these manifestations may persist for a considerable time in very slight cases they may be absent altogether

(5) The signs of peripheral neuritis are very variable in many outbreaks the knee jerks may be absent in more than half of the cases over long periods of time, in other outbreaks exaggeration of the knee-jerks may be the rule in most outbreaks the jerks are exaggerated in some patients absent in others and even in the same person they may at times be exaggerated and at other times diminished or lost In connection with the peripheral nervous system there are physical signs and symptoms which suggest that the nerves are acted on by a neuro toxin which produces irritation or degeneration, or both

(6) In severe attacks there is often a pronounced tendency to hæmorrhages bleeding piles epistaxis hæmatemesis or even hæmorrhagic retinitis may occur and a blotchy erythema of the skin of the swollen parts is often noticed this has been described as a rash by some observers

(7) There is a great tendency to glaucoma during the later stages of the disease

(8) There is a liability to sudden death from heart failure

(9) One attack does not confer immunity, but on the contrary predisposes to further attacks

(10) The incubation period is very variable as a rule the disease appears within a short time after exposure to the causal agency I have seen a case in which the disease began within a week of arrival in the affected family many cases have been reported in which symptoms have appeared within one or two days of exposure to the causal conditions

(11) Convalescence is prolonged after a severe attack but it is usually rap l when the case has been properly handled from the outset of the disease

Taking these features of the disease into consideration the most reasonable explanation is that *under certain conditions stored parboiled rice may become toxic* The poison by ingestion causes first a gastro intestinal irritation on absorption it causes damage to the peripheral nerves including those of the cardio vascular system also to the blood vessels and probably to a lesser extent to the entire active tissues of the body *The poison is probably often present in stored rice in* such small quantities as to give rise to no obvious symptoms under certain conditions it occurs in larger amounts and causes disease manifestations of varying intensity The sub effective poison is probably important in that it predisposes to the disease so that *those who live regularly on stored parboiled rice* are liable to show symptoms from doses of the poison which would be insufficient to affect other persons whose rice had previously been non toxic It is quite possible that stored parboiled rice may often be toxic but the quantity of poison may be insufficient to cause symptoms

Is Epidemic Dropsy a form of Beri beri?

If we accept the orthodox definition of beri beri as a disease caused by deficiency of vitamin B in the diet the answer *will* be No but if we regard *beri* beri as a *name* which has been employed to designate cases of disease which show certain manifestations the negative answer cannot readily be given

Many medical men are impatient with the sceptics who persist in doubting the orthodox doctrine with regard to beri beri The weight of authority is on the side of men like Vedder *who hold that vitamin deficiency has been so firmly* established as the cause of beri beri that all other theories have died a natural death But there are still many obstinate persons including McCarrison Noel Bernard and some Japanese authorities who persist in expressing doubts and I am on the side of the heretics although I do not agree with all their views

If we admit that we do not completely understand beri beri it is permissible to make a broad general survey of the position Under the name beri beri various disease manifestations have been included, the variations may be explained in one of two ways (1) that one causal agency is at work but with varying intensity and over varying periods of time just as alcohol for example may produce acute gastric symptoms chronic neuritic symptoms cirrhosis of the liver etc (2) that more diseases than one have been included under the term beri beri

It is also quite possible that both of these views may be true and that one of the diseases called beri beri may be very variable in its manifestations while there are also other distinct diseases which have been included under the name beri beri If we start from the reasonable assumption that one general type of causal agency may have variants and so can produce a variety of symptoms, it may be possible to include several forms of beri beri in one group and so we shall avoid the complications involved by the use of a multiplicity of disease names The beri beri of the Far East is well known to be protean in its manifestations in the same outbreak there may be dry neuritic cases and pronounced dropsical cases and these forms may alternate even in the same patient Let us look for a moment at the broad *differences* which are stated to exist between epidemic dropsy and the classical types of beri beri These are —

(1) Epidemic dropsy occurs among the eaters of parboiled under milled rice while beri beri is essentially a disease of those who eat over milled rice

We may omit for the moment the problem of the possible rare occurrence of a beri beri like disease among those who do not eat rice at all as this would involve a prolonged discussion in itself

(2) Fever and gastro intestinal symptoms are much more conspicuous features of epidemic dropsy than of beri beri, but they have also been emphasized as features of beri beri by certain competent observers so that they cannot possibly be employed as a means of distinguishing between the two diseases

(3) Hæmorrhages are common in severe outbreaks of epidemic dropsy they are not common features of beri beri of the Far East though they are conspicuous in 'ship beri beri'

(4) Glaucoma is common in epidemic dropsy, I am not aware of its occurrence in the beri beri of the Far East

(5) Infantile epidemic dropsy has not been observed while beri beri of sucklings is a well recognized condition in some places in the Far East

(6) Peripheral neuritic manifestations are more conspicuous features of the beri beri of the Far East than of epidemic dropsy though they are quite as pronounced in some cases of epidemic dropsy as they are in the cardiac forms of beri beri

These are the most important respects in which differences occur

The points of *resemblance* are —

(1) Both diseases affect rice eaters the exceptions to this general statement are so rare and so doubtful that they do not invalidate the broad generalization

In both cases it is essentially the eaters of manufactured stored rice who are attacked

(2) The seasonal distribution of both diseases is remarkably similar

(3) The age distribution is the same in both if we leave out of account the beri beri of sucklings

(4) Both diseases are specially liable to occur as family or institutional outbreaks and among people who are eating rice from the same source of supply

(5) Person to person conveyance of the disease can be excluded in the vast majority of outbreaks of both diseases

(6) Exclusion of rice from the diet controls outbreaks and attacks of both diseases

(7) Both diseases are very variable in severity and in symptomatology

(8) The same type of cardiac manifestations is common to both sudden death from cardiac failure is also a frequent occurrence in both

(9) The œdema is of the same type in both

(10) The nerve degenerations are of the same general type

(11) Gastro intestinal disturbances at the onset are frequently seen in both diseases

(12) The course of the diseases and the slow convalescence are remarkably similar

(13) The post-mortem findings have not been shown to be different in any essential respect

(14) *Exactly the same theories as to causation have been advocated in both* diseases (*a*) rice intoxication (*b*) vitamin deficiency (*c*) infection (*d*) toxi infection The only exception to this statement is that mustard oil poisoning has been suggested as a cause of epidemic dropsy, but for this view there is no satisfactory evidence Taking a broad view of the diseases it may be asserted with some confidence that any ordinary outbreak of epidemic dropsy would be called beri beri if it occurred in the Far East while cases of the cardiac form of beri beri such as were recently described by an observer in the Philippines would be diagnosed as epidemic dropsy if they were seen by a Calcutta physician Even if this assertion be accepted it does not settle the question as to whether the diseases are the same certain differences still exist and the point is whether these differences are of degree or of kind

If both diseases were due to a poison which is produced in rice by the action of a microbe they might still be different in type owing to the known differences which exist in the kind of rice concerned A microbe growing on parboiled rice might well produce *different poisons from those produced by the same* microbe acting on polished rice Different microbes may, of course, be concerned

If the differences between the diseases were accounted for in either of these ways we should have no justification for giving them different names though we might well adopt some distinctive sub titles to indicate the variations which occur

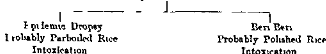
either in symptomatology or ætiology. In connection with the variations which occur in beri beri and epidemic dropsy, reference has already been made to the influence of the dose of the causal agency and the period of time over which it acts.

Does vitamin deficiency play a part in beri beri? You may have formed the impression that my opinion is dead against vitamin deficiency playing any part in the causation of beri beri. As far as epidemic dropsy is concerned there is little or no evidence that vitamin deficiency has anything to do with the disease and though I am less competent to express an opinion on the subject of beri beri, it appears probable to me that those forms of beri beri which are most nearly related to epidemic dropsy are likely also to be caused by rice intoxication.

But there are two considerations which appear to be of possible importance in this connection, one is that vitamin deficiency in the diet would certainly lower the resistance of the body to the action of any poison so that a dose which would be ineffective to a healthy person might readily produce symptoms in a person whose vitality has been lowered by a deficient diet. The second consideration is a more direct concession to the advocates of the deficiency view, it is that there may well be an actual disease in man which is caused by deficiency of vitamin B in the diet and if such a disease does occur it would certainly be diagnosed as beri beri. The chief feature of this disease would be a chronic degeneration of the peripheral nerves but neither dropsy nor cardiac enlargement would be expected to occur, also we should expect to be able to account for the symptoms by an enquiry into the dietary. In the past those who have discussed beri beri have been too ready to adopt the unitarian view: they have assumed that beri beri is one disease and when they have discussed its ætiology they have regarded the name beri beri as a sufficient description of the disease. Repeatedly in the literature we find reports of outbreaks in which no details are given of the symptoms of the patients and we are merely informed that certain numbers of people suffered from 'beri beri.' In my opinion writers ought to state what symptoms were present as two outbreaks which are called beri beri may well be outbreaks of two distinct diseases, the one being a deficiency disease and the other a rice intoxication.

It is possible that we have been led astray by accepting the suggestion that the name beri beri is a designation of a disease. Here is a provisional classification of the diseases which have been called beri beri:—

I. The Beri Beri Group or Rice Intoxication Disease



II. Avitaminosis B or Polyneuritic Avitaminosis.

In addition to these three types of disease of which the first two in my opinion have the best right to be called beri beri, we may well have a combination of avitaminosis B and rice intoxication as persons who live chiefly on a diet of polished rice are likely to suffer from rice intoxication as well as from vitamin B deficiency.

A further complication is likely to exist, those who have tried to demonstrate the production of beri beri in human beings by feeding prisoners on a diet which was deficient in vitamin B had great difficulty in devising a suitable dietary for their experiments Their test diet in fact was seriously defective in calories in available proteins and in palatability, so that the results of the experiments were decidedly unconvincing Some of the prisoners who were kept on the control diet rich in vitamin B suffered more than some of those who were fed on the diet deficient in vitamin B although on the average those who were kept on the deficient diet naturally showed greater nutritional disturbances than the controls

In any case it is rare to find a diet whose only defect is in vitamin B I can well imagine my hearers protesting against this complication of the picture Not merely do I suggest that there are two chief forms of rice intoxication but that vitamin B deficiency is likely to complicate these and further that vitamin B deficiency is likely to be complicated by other defects in the diet

But let me point out that the complication is not of my making You must blame Mother Nature and you cannot even throw the blame entirely on Her human beings have not been content merely to cool the rice which Nature has provided they have first of all killed it and milled it and then kept it in this mutilated condition for months exposed to the assaults of bacteria and moulds which have been aided by moisture and heat and darkness Under such conditions we must expect complications and confusion to result and we cannot get rid of the consequences of our actions merely by uttering the blessed word Avitaminosis I will even admit that my own incantation '*rice intoxication*' may not be sufficient to explain everything

My discussion up to the present has aimed at suggesting that we have still much to learn about beri beri and that further intensive work will have to be done before we understand all the mysteries of the problem But if I have been critical as to our achievements in the direction of finding the cause of beri beri I can strike a much more hopeful note when it is a question of controlling the disease I believe that we have acquired enough knowledge to enable us to prevent all the diseases which have been called beri beri These diseases have never been known to arise when the following conditions have been fulfilled —

(*a*) When rice has been properly stored in a dry condition in the form of *paddy* manufactured in a suitable way preferably by parboiling, and then stored after thorough drying in a dry well ventilated store room, and

(*b*) When the food contains a sufficient amount of vitamins and all the other constituents of a satisfactory diet

You will see that I do not allow any preconceived ideas not even my own to interfere with the locking of both doors of entry of the diseases under discussion the one door is locked against any possible poisons which may be formed in the rice and against the damage of other kinds which result from improper storage the other door is locked against injury to the body by dietetic deficiencies

To my mind it is criminal not to advocate the locking of both doors and it seems to me that when both have been locked, the arguments as to the nature of beri beri and epidemic dropsy will become pleasant academic discussions instead of being heated controversies in the presence of a dangerous enemy

THE ANALOGY BETWEEN THE BERI BERI DISEASE GROUP AND PELLAGRA

Although I have no personal knowledge of pellagra I have been much impressed by the analogy which appears to exist between that disease and beri beri

Just as beri beri may be stated broadly to be a disease of rice eating people, so pellagra with rare and doubtful exceptions is a disease of corn eating people

The first descriptions of pellagra appeared just about the time that corn was introduced to Europe as an article of food In Roumania the appearance of the disease was associated with the importation of wet and damaged corn by coasting vessels In England the cases which have been closely investigated have been found to be associated with the consumption of large quantities of corn flour

The theories which have been advanced to explain the causation of pellagra have been just the same as those which have been advocated in the case of beri beri Casal and Lombroso long ago maintained that spoiled maize was the cause Infection available protein deficiency and vitamin deficiency have been warmly supported at a later date

The course of events in pellagra is not unlike that of beri beri, gastro intestinal disturbances are pronounced at the outset then come tropho neuritic symptoms so that there is a strong suggestion of the swallowing of a toxin which first of all causes irritation of the gastro intestinal mucosa and then is absorbed causing damage to the nervous system There is definite evidence of neuritis fleeting pains paræsthesiæ tremors exaggerated reflexes and later on contractures may occur The most successful treatment lies in a good diet from which corn has been rigidly excluded

Both pellagra and beri beri have the same type of seasonal distribution both diseases are rare in young children both diseases tend to pick out groups of people living on the same diet Although the point has not been specially enquired into, it would appear from the records that pellagra like beri beri is essentially a disease of people who live on a grain which has been stored in bulk and that its incidence tends to be explosive A broad survey of the two diseases in which special enquiry is made into the general conditions of diet of the affected persons might yield results of value and might lead to a serious reconsideration of the views of the old masters who held that both diseases were associated with unsuitable conditions of storage of the grains which form the staple articles of diet of the persons who suffer from the diseases

The work on the vitamins has not been wasted it has emphasized an imported factor in nutrition, but perhaps vitamins have been overdone and have been saddled with an undue share in the responsibility for two very important and very intriguing diseases

SUMMARY

Epidemic dropsy shows many points of resemblance to certain forms of beri beri The chief features of epidemic dropsy are described and compared with those of beri beri

A scientific definition of beri beri cannot yet be given the name may have been applied to two or more distinct diseases one of which is probably closely related to epidemic dropsy

Reasons are given for the belief that epidemic dropsy is caused by poisons which are produced by the action of a microbe in parboiled rice which has been kept in hot and damp stores One form of beri beri, probably the more important one is likely to be caused in the same way except that the poison is produced on over milled rice

A disease caused by deficiency of vitamin B in the diet probably occurs this is likely to be confused with beri beri but it lacks some of the important features of that disease

The points which still require elucidation in connection with beri beri disease group are referred to A plea is made for the adoption of simple preventive measures which will ensure protection against all the diseases which have been called beri beri

Pellagra shows many points of analogy with beri beri it may be worth while to make a comparative study of pellagra and beri beri with special reference to the part played by storage of corn and rice

BERI BERI COLUMBARUM

BY

LIEUT COL R McCARRISON C I E M D D SC LL D F R C P I M S
Director Deficiency Diseases Inquiry Indian Research Fund Association

THERE are three elements in beri beri—the multiple neuritis the cardiac derangement and the oedema—each as important and as characteristic of the disease as the other Pathologically the disease is characterized (1) by degenerative changes in the entire nervous system particularly the peripheral nerves the cord the vagus and the sympathetic system (2) by diffuse parenchymatous degeneration of the muscles combined at times with hyaline and fatty degeneration (3) by hypertrophy and dilatation of the heart most marked on the right side with fine fatty degeneration and fragmentation of its muscle fibres (4) by oedema and serous effusion anasarca hydropericardium hydrothorax and ascites one or other or all of which may be present in the same case (5) by chronic passive congestion of the abdominal viscera especially the liver spleen and kidneys and (6) by congestion of and ecchymoses into the mucosa of the stomach and duodenum (Byam s System of Tropical Medicine) To these is often added enlargement with increased epinephrine content of the adrenal glands The combination of pathological findings occurs in no other disease but beri beri

An attempt was made to reproduce this pathological picture in pigeons by means of diets containing an insufficiency of vitamin B This attempt was successful The diseased state is referred to as *beri beri columbarum* to distinguish it from *polyneuritis columbarum*

The investigation in which 1 87- pigeons were used involved the study by statistical methods of the normal range of variation of the heart weight as compared with body weight in normal pigeons so that standards might be available for the recognition of cardiac hypertrophy in the deficiently fed birds and the subjection of the experimental data to statistical examination

The results of the investigation full details of which are to be found in *Indian Medical Research Memoirs* No 10 were as follows

(1) A condition—*beri beri columbarum* having all the pathological characters of human beri beri has been produced under experimental conditions in pigeons by means of diets of similar composition to those in use by human sufferers from the disease This condition differs from *polyneuritis columbarum* in certain regards of which hypertrophy of the heart is the chief

(2) The basal factor in the production of *beri beri columbarum* is insufficiency but not complete want of vitamin B (or of the anti neuritic fraction of this vitamin)

(3) There is an optimum degree of insufficiency of vitamin B at which *beri beri columbarum* is most likely to arise this optimum is provided by diets of which the vitamin B value is from 30 to 50 per cent below the minimum required for the maintenance of normal metabolism

(4) The ultimate cause of the disease is not the *negative* factor of vitamin insufficiency but a *positive* and toxic agent produced in the course of a disordered metabolism arising out of the vitamin insufficiency The clinical and pathological manifestations of beri beri are due in whole or in part to this specific agent

(5) The existence of this agent has been demonstrated on pathological grounds and by statistical examination of the experimental data

(6) *Beri beri columbarum* and *beri beri hominum* are preventable by the same means

(7) Since *beri beri columbarum* can be produced by diets of similar composition to those in use by the subjects of *beri beri hominum*, since the pathological features of the two states are to all appearances the same, and since both are preventable by the same means it is inferred that the aetiology of the two conditions is the same

(8) Experiments on animals suggest that beri beri like maladies may be of different kinds some due to infectious causes, some due to dietetic causes and some due to a combination of both The variety of 'beri beri' dealt with in this communication is due to dietetic causes only, it is believed to be the variety which is endemic in certain parts of the Madras Presidency

(9) *The results of this investigation confirm the generally accepted view that* endemic or true tropical beri beri is due to insufficiency of vitamin B and not to toxic substances produced in rice by bacterial action

The mechanism of beri beri production in pigeons is as follows —

A diet of polished rice rarely causes true beri beri in pigeons but if with this diet each bird be given 0 8 gramme of *dhal arhar* daily—a legume commonly used by rice eating peoples—then true beri beri occurs in approximately 25 per cent of birds so fed Similarly if a well balanced diet of mixed grains be autoclaved at 130° C for two hours in an alkaline medium and its vitamin B content *reduced* to the requisite point then beri beri likewise arises Assuming that the figure 100 represents the amount of vitamin B required in any given diet to keep pigeons at maintenance level without the occurrence of polyneuritis then diets containing an amount of this factor which is below 100 and is represented by figures lying between 50 and 70 will give rise to true beri beri Any further reduction in the vitamin content of the diet to below 50 will tend to cause *polyneuritis columbarum* (which is a specific form of starvation incompatible with life) rather than *beri beri columbarum* For the production of the latter disease a little vitamin B is necessary and the degree of vitamin insufficiency must be just so *(50 to 70)* The

disease can therefore be produced either by adding an amount of *dhal* to a diet of polished rice which will *raise* the vitamin value of the diet up to between 50 to 70 or by autoclaving a vitamin rich diet so as to *reduce* its vitamin value to this level of insufficiency

Rices such as lightly milled or parboiled rices which have of themselves a 'vitamin value' of from 50 to 70 will similarly produce the disease when fed exclusively to pigeons Such 'beri beri producing rices,' which were also very deteriorated have caused the disease in pigeons but the birds were *completely* protected from it by the addition to the deteriorated rice of *dhal* or wheat in amounts sufficient to raise the vitamin value of the diet to 100 or over thus demonstrating that *beri beri columbarum* is not due to a poison in the rice itself Beri beri has been prevented in a certain jail in Burma by this means, as an experimental procedure in man thus confirming the observations on pigeons

DISCUSSION

Lieut Col Edward B Vedder (Philippine Islands) I have had no experience with epidemic dropsy but have always objected to the assumption of a hypothetical toxin to explain the incidence of beri beri or epidemic dropsy With regard to the latter if the rice is toxic such a toxin should be demonstrable just as botulinus toxin from food on which *B botulinus* has grown is readily demonstrable It has always seemed to me that the theory that beri beri is caused by several deficiencies is more plausible According to this theory dry beri beri and *polyneuritis columbarum* are caused by the deficiency of an anti neuritic vitamin which is one constituent of the vitamin ordinarily called B Wet beri beri epidemic dropsy and possibly war œdema and œdematous diseases occurring among peoples subsisting on white bread and diets other than rice are according to this hypothesis caused by a second deficiency which may be a vitamin or some other unidentified food principle Beri beri can be cured by the administration of extracts of rice polishings without changing the diet of the patient a fact that strongly indicates that beri beri is not due to an intoxication I should like to ask Col Megaw if he has ever similarly tested the theory that epidemic dropsy is caused by toxic rice If the disease can be cured whilst continuing the supposed toxic rice, it would point towards a deficiency rather than a toxin in the rice I would like to emphasize the point that the differences of opinion between Col Megaw, Col McCarrison and myself are all on scientific points and that there is no serious difference with regard to the proper measures to be taken to prevent the incidence of beri beri or epidemic dropsy

Dr B Shaha (Bengal) In the speaker's opinion epidemic dropsy is of infective origin for the following reasons

(*a*) In a multimillionaire's house in Calcutta there were two separate establishments for food One for the master and his family upstairs and the other for the servants and officers downstairs The same rice and mustard oil were used in both the kitchens The master and his family although having richer food suffered and the servants and officers escaped

(*b*) One convalescent of epidemic dropsy from Calcutta went to Natore as superintendent to a Raja, who, in a month's time with his whole family fell victims to

the disease They used *excellent rice, fresh milk, plenty of fish and there was no other* case in the vicinity The superintendent's father in law and mother in law came from ten miles to attend on them and they on their return started the disease in their family

(c) In the Medical College Hostel there were several messing establishments in the same building *with the same rice and oil, and yet some of the messes suffered* and others escaped

(d) British Troops in Mesopotamia, as is reported in the official History of War, who never touched rice suffered from dropsy in epidemic form Some thought infected meat was the cause

(e) *In east Bengal, where rice is taken for breakfast, the midday meal the evening* meal and supper i e four meals, we have never seen an indigenous case of epidemic dropsy but only imported cases from town We have to remember that in that part the upper classes and upper middle classes do not take the coarse variety of rice which is the only kind cultivated over there They import fine, husked parboiled rice, one year s consumption at a time i e their rice is exactly similar to that of Calcutta

(f) Resume of symptoms Initial temperature, sometimes very high, as much as 104° 105° F Diarrhœa and dropsy, which is not cardiac or renal in origin limited to the legs ending in some cases with severe cardiac failure of the congestive type (relieved by leeches and massive doses of digitalis) and an erythematous eruption limited to the legs In some cases big nœvoid eruptions suggest an infective cause although we have failed so far to demonstrate its presence in spite of the vigilant care of the research workers

Finally one might mention that the cases with very high temperature did not show any blood changes, such as a leucocytosis or the presence of any parasite, to explain the high temperature This temperature was easily controlled by the daily intravenous injection of 1 to 2 c cs of iodine solution the formula being, Iodine, gr ix, Potas Iodide gr xviii and aq dist oz ii, kept in a stoppered phial as a stock solution

Major G G Jolly I M S (Burma) In Burma we have frequent outbreaks, usually small of beri beri which we have come to associate with bad storage We fully realize the importance of the dry ventilated store room ' mentioned by Col Megaw as a preventive but the difficulty in obtaining it has been great mainly owing to the fact that the outbreaks generally occur when the rains are well established and the humidity high and that the rice used has to be carried long distances

I understand that some of the inhabitants of Bengal store their rice in lime I would like to ask Col Megaw whether he has any knowledge of beri beri or epidemic dropsy occurring among persons who store their rice in this manner

Dr C A Bentley (Bengal) Inquired whether a detailed dietary of patients suffering from epidemic dropsy for some considerable time prior to the onset of the disease could be supplied He considered that in all probability the disease exhibited a number of different forms

R B Dr Chuni Lal Bose (Bengal) I wish to make a few observations on this subject based on my experience It was 40 years ago that I first saw a number of cases of epidemic dropsy in a jail in Upper Burma There were about 30 prisoners in the jail and 50 per cent were attacked The signs and symptoms were similar to what

we find in the present day cases in Calcutta and other parts of Bengal They had œdema of the lower extremities dilatation of the heart shortness of breath on slight exertion tingling and numbness in the lower extremities accompanied with tenderness in the calves on pressure and followed by marked loss of power of locomotion feeling of oppression in the chest and of burning in the stomach sometimes attended with diarrhœa Some prisoners died of the effects of the disease and I had an opportunity of making post mortem examinations with Major Wright I M S The only pathological changes noticed were general congestion of the venous system congestion of the thoracic and abdominal viscera and dilatation and engorgement of the right side of the heart The diet allowed to these prisoners was exactly the same as they used to take at home viz rice fresh meat, plenty of green vegetables and a quantity of *gnapi* (rotten fish) The vegetables and meat were cooked with gingelly oil Col Megaw has thrown out a suggestion that next to rice mustard oil might be investigated as a causative factor in epidemic dropsy My object in bringing up this epidemic among the Burmese is that *mustard oil does not appear to play any part in the causation* of the disease The Burmese do not use mustard oil at all as we do in Bengal There is another fact regarding mustard oil which I wish to bring to your notice Epidemic dropsy has on many occasions occurred among the Marwari people in Calcutta and Howrah but the Marwaris do not use mustard oil in the preparation of their food They use *ghee* only These two facts go against the theory that mustard oil may be a causative factor of epidemic dropsy

From my knowledge and experience of a large number of cases of epidemic dropsy I am inclined to support Col Megaw that some kind of toxin produced in rice is responsible rather than that it is a vitamin B deficiency disease Cases occur *among* very well to-do people whose diet is rich in all kinds of vitamins besides rice and some of them have shown very bad types of the disease and have died of it Supposing the particular rice taken by the victims was deficient in vitamin B why should they suffer from epidemic dropsy if they eat other articles of diet rich in this vitamin? *The disease is more a disease of towns than of villages This fact also* supports Col Megaw's theory of a toxin Those who know anything of the life of our village people in Bengal know full well that they as a rule never store rice (only paddy) for future use, and they prepare a small quantity of rice at a time for immediate use Such rice cannot possibly get infected in such a short time in the same way as rice stored for long would do

Lieut Col T H Gloster I M S (Madras) With reference to Col McCarrison's remarks on the bacteriological work referred to in his paper I isolated and studied 35 bacilli from the rices used by him in his feeding experiments Twenty four belonged to the mesentericus group and of these 9 were identified as *B vulgatus* and 7 as *B mesentericus* 6 of these were facultative anaerobes which closely resembled *B mesentericus* type A found by Lloyd Clark and McCrea to be the commonest organism producing 'rope' in bread Other bacilli found were *B megatherium* *B cereus* *B laterosporus* *B terminalis* and *B circulans* None of these bacilli were *only* found in rices which were associated with beri beri and in general, their distribution among the rices examined does not suggest that any of them have a causal relation to the disease

Feeding experiments were carried out with cultures of all these bacilli in a rice medium Both cultures which had been incubated aerobically at 37° C for seven days and cultures which after a preliminary incubation at 37° C for three days were covered with paraffin and kept for a further seven days at 50° C were used Guinea pigs were fed daily for 21 days with the cultures which were mixed with their bran Such guinea pigs received a complete culture (about 10 c cs) daily Their diet consisted *of wheat bran gram and green food None of the animals have shown any symptoms* of paresis and their general health has remained good These are still, however under observation

Lieut Col C A Sprawson I M S (United Provinces) Agreed with Col Megaw as to nature of beri beri and epidemic dropsy and as to the existence of two factors a vitamin deficiency and a toxin from food storage, both of which may operate singly or together He directed attention also to Aden where no rice is eaten by British troops only wheat which has hitherto been stored for six months or more In Aden a constantly occurring series of polyneuritic cases had been admitted during the last 30 years It is suggested that with the abolition of such prolonged storage of flour the series of cases may cease

Prof J I Rosedale (Straits Settlements) In support of Col McCarrison's remarks we have been able to produce symptoms akin to beri beri, but distinct from the usual head back polyneuritis, in pigeons by feeding cooked polished rice to which has been added an amount of minerals sufficient to bring the content of the white rice up to the mineral content of white flour

We have produced from rice polishings two fractions of our extract one of which (the non fermentable) relieves the beri beri condition referred to For the cure of the beri beri symptoms in the presence of avian polyneuritis both fractions are required *The figures of Plimmer and Rosedale support Col McCarrison s observations that* a certain level or balance of protective food is required

Major H Stott I M S (United Provinces) With regard to the relationship of beri beri and of epidemic dropsy my interest in this subject began in 1911 when I met with an outbreak of beri beri in Madras Amongst these cases were some of dry beri beri, some of typical wet beri beri and some mild cases of wet beri beri with fever which were of interest I came to Calcutta and discussed with Captain Megaw, as he then was cases of epidemic dropsy in Calcutta, and on my return I felt then and my further clinical experience has made me feel since, that beri beri and epidemic dropsy cannot be separated but should be included as members of one comprehensive beri beri disease group

In considering whether the beri beri group is a deficiency disease or due to toxemia clinicians should not be mesmerized by laboratory investigations however scientifically they have been performed when they find that those investigations are out of accord with clinical facts or out of accord with the outcrop of cases in epidemics I do not deny that some cases of the beri beri group may be due to a deficiency, but I do deny that all cases of the beri beri group, so far as my experience goes, are due to a deficiency There is some sound evidence that there is a toxic cause and that this toxin is nearly always in stale rice In the outbreaks of beri beri amongst British soldiers that I have seen (e g , in Lebong) there has been no question of any deficiency

of vitamin B or of other substances in the diet which has been ample and varied, and yet beri beri has occurred amongst them Col Megaw Col Sprawson and Dr Bose have given us other examples I find it difficult to accept Lieut Col Vedder's suggestions that there may be two separate vitamin deficiencies to account for beri beri and epidemic dropsy for such cases in my opinion belong to the same beri beri group and are probably due to the same cause Lieut Col Jolly asked for information about the prevention of beri beri by adding lime to stored rice Such an experiment was conducted in the jail adjoining the Presidency General Hospital Calcutta some 15 years ago and was I believe entirely successful

Lieut Col R Kelsall I M S (Burma) There are only one or two points that I should like to make in this discussion and I rise curiously enough for the reason that I have seen very little epidemic dropsy but a good deal of beri beri Some speakers, have tended to regard the two as the same disease and I admit that in Calcutta where you see a good deal of beri beri and also of epidemic dropsy it may be difficult clinically to say where one disease ends and the other begins If you put a case of beri beri with œdema in one bed and a case of epidemic dropsy in the next I admit that it will generally be very difficult to make a diagnosis between them Our experience however is different in Burma I personally have known beri beri there for over 20 years and in the Rangoon General Hospital we always have many cases of beri beri We know the disease in its ordinary clinical manifestations But about three years ago in Rangoon we were suddenly confronted with an outbreak of epidemic dropsy To those of us who were working in Rangoon this was a disease we had never seen before and from the clinical point of view was a new disease Although as I say, it might be impossible to take any individual case, and say whether it was beri beri or epidemic dropsy, yet this sudden outbreak of cases all of the same type more explosive in character than anything we had ever seen in the case of beri beri and occurring amongst a different class of the population was to all of us working in Rangoon very suggestive of the diseases being different

Further, as has been stated by previous speakers the incidence of the disease made it very difficult in one epidemic to ascribe it to a food deficiency I remember one family of well-to-do Bengalees, in which the disease attacked everybody in the family including the servants, within about 14 days The dietary was exceptionally good the whole family partaking daily of fish meat, fruits vegetables eggs and milk in addition to their rice It seems very difficult to reconcile any theory of vitamin deficiency, which, one would think, must be of slow development with an explosive outbreak of disease like this in an apparently thoroughly well fed family

To my mind the incidence of the disease as I saw it had very much more the appearance of an acute infectious disease On the question whether the infection may possibly have arisen from infected rice or not I am not able to venture an opinion

Dr S C Dutt (Bengal) Adulterated food aggravates the condition of epidemic dropsy Polished rice or bad rice do the same thing as also will adulterated mustard oil

Dr V G Heiser (U S A) It is very gratifying to see by the large numbers present that there is an ever increasing interest in food deficiency as an important factor in the causation of disease There is now no serious objection to the view that certain

diets commonly used in the Orient are responsible for disease because they do not contain certain food factors While the research workers are finding out just what deficiencies or toxins produce a particular series of symptoms let us see that practical steps are taken to have more adequate diets used among people whose staple article of diet is rice and fish for instance it has been found by experiments tried on a large scale over and over again that if the rice in the diet is undermilled beri beri will disappear This being true is it not a reflection that over a 100 000 people die in the Orient every year and over a million are made ill? This Congress should again take action to bring this state of affairs to the notice of the various countries concerned

Lieut Col R McCarrison I M S (B India) replied Col Vedder has raised objection to my assumption that in the form of experimentally produced beri beri with which I have been dealing I have supported the view put forward by Eijkmann in 1896 that the ultimate cause of the disease is a toxin produced in the body in the course of a disordered metabolism arising out of vitamin insufficiency This is of course merely an hypothesis but I venture to think it is a good working hypothesis the existence of such a specific toxin is suggested not only as a result of statistical study of the material, but on pathological grounds for the appearance of the heart is characteristic resembling closely that seen in diphtheria in which we know a toxin is the cause of the heart changes Arguing therefore, from analogy we suspect the existence of a toxin however it be produced which exercises a specific action on the heart in *beri beri columbarum*

I have derived the impression from this discussion that we have not been arguing about the same disease I do not know whether or not epidemic dropsy is the same disease as beri beri *All I have endeavoured to do is to bring forward the evidence that* a condition can be produced in pigeons by dietetic means which possesses all the pathological features of a malady which we are accustomed to include under the generic term beri beri This experimentally produced condition is due to an insufficiency of vitamin B There is therefore at least one type of disease to include under this generic term which is a deficiency disease whatever be the cause of ' epidemic dropsy

I would add that what we need most in this country at the present time is the clinical pathological and dietetic definition of the maladies included under the generic term ' beri beri Until this is done we will continue to confuse one with the other and to argue at cross purposes This much however appears to be certain that a properly constituted diet rich in vitamin B will prevent this group of diseases and this knowledge we owe to the experimental method and to experiments on pigeons despite that the pigeon is not the same thing as a man nevertheless man desirous of escaping beri beri like maladies owes much to the pigeon As with food so with mental processes *balance* is essential

Lieut Col J W D Megaw I M S (Bengal) in reply Apologized for the obvious shortcomings of his paper these were inevitable because of the impossibility of dealing with so wide a subject in a short paper He also expressed some surprise that so many of his critics were prepared to accept views which for many years had been regarded as heretical Replying to Col Vedder s criticisms he pointed out that war œdema was

different in many essential respects from epidemic dropsy in war œdema there is bradycardia and polyuria but most important of all, the previous diet of the victims had always been of such a nature that the occurrence of the disease could be predicted and explained from a knowledge of the diet alone This was not the case in epidemic dropsy for, as a rule, no fault could be found with the diets of the persons attacked by that disease

Col Vedder had pointed out that the toxin had not been detected on this point Lieut-Col Megaw referred to the work of Acton and others, but expressed no opinion as to whether the toxin discovered by them was that of epidemic dropsy He also said that if the toxin were not regarded as having been discovered neither had the vitamin deficiency been demonstrated, so that it became a case of choosing between a hypothetical toxin which fitted in with the known facts and a hypothetical vitamin deficiency which did not fit in with the facts

Treatment by extracts of food grains had not been tried because all the early cases responded very promptly to a rice free diet in which there was a liberal supply of available animal proteins fresh milk being the best of all Good results would doubtless follow from the use of extracts but they could not be better than those from a good wholesome diet with plenty of fresh milk In the great majority of cases the tendency was towards spontaneous recovery even when no change had been made in the general composition of the diet

Replying to Dr Shaha he asked for evidence of the person to person communicability of the disease There were one or two recorded instances of the occurrence of cases of the disease in certain localities soon after the arrival of persons suffering from the disease these were exceedingly rare and in every known case they occurred in places in which the disease was known to be liable to occur as an endemic disease In hundreds of cases in which patients from Bengal had gone to Bihar and the United Provinces there was no evidence that the inhabitants of these places ever contracted the disease from affected persons though the point had been very carefully investigated and many doctors from these localities had reported that they had never come across any case in which infection had spread from affected persons

He was rather surprised to hear that many of the people of eastern Bengal obtained their supply of rice from Calcutta and he would like to have some detailed information on the point

Dr Bentley had asked for a detailed account of the diet of the affected persons for a long period before the onset, such an examination of the diet could of course not be obtained, but the diets had been very carefully enquired into Col Megaw asked if it was conceivable that a series of explosive outbreaks such as were illustrated in the charts which were shown, could possibly originate from a diet deficiency especially as some of the most severe family outbreaks had occurred in families whose diets had been exceptionally good in every observable respect He agreed with Dr Bentley as to the probable existence of many minor forms of the disease These were referred to in the paper

Col Sprawson referred to cases in which there was no rice in the diet, but only wheat flour which had been stored it was quite possible that the disease might originate

from poisons formed in other grains besides rice, and it was important that the storage of all kinds of grain should be attended to, indeed he hoped that the word 'storage' would be firmly impressed on the minds of everybody who was interested in the important question of the diets of people in the tropics Far too little attention had been paid to this important matter

It was true that as a broad generalization, the disease was associated with the eating of rice more than 99 per cent of the cases occurred in rice eaters and the rare occurrence of the disease in persons who were believed to have eaten no rice might possibly be explained by adulteration of wheat flour with rice Col Kelsall was satisfied that *epidemic dropsy was a different disease from beri beri but more evidence* would have to be produced as epidemic dropsy was not always explosive while beri beri was sometimes explosive It would be quite possible to pick out single outbreaks of beri beri and of epidemic dropsy which appeared to be very different from each other, but when we study all the recorded outbreaks, the distinctions break down and every grade of transition between the two diseases could be shown to occur hence the difficulty in regarding the two diseases as distinct from each other

Major Stott had already dealt with Major Jolly's question about the use of lime as preservative of rice This point had been referred to by him (Lieut Col Megaw) in his paper on the 1909 outbreak in Calcutta In this paper it was shown that the drying of stored rice in the sun and its treatment with lime had protected the prisoners in the *Presidency Jail from epidemic dropsy when the disease was almost universal* among the rice-eating people of Calcutta

The important evidence brought forward by Rai Bahadur Dr Chuni Lal Bose was most valuable Lieut Col Megaw did not believe that mustard oil was the cause of the disease he merely referred to mustard oil as being worthy of fuller investigation than it had hitherto received

Col Gloster referred to beri beri producing rice, Lieut Col Megaw asked for caution in accepting any samples of rice as being responsible for producing an outbreak which was under investigation as a rule it was impossible to obtain actual samples of the rice which had been associated with the outbreak On one occasion the workers at the Calcutta School had nearly succeeded in obtaining a sample which was obviously *concerned but unfortunately the rice dealer, who had sold this sample,* succeeded in getting possession of it and took steps to dispose of it so that it could not be secured for experiment

He also pointed out that it was not enough to obtain a sample of rice from the same source as the offending rice two bags from the same consignment might be exposed to very different conditions and so they could in no sense be regarded as being the 'same rice

In concluding he expressed his regret that limitations of time made it impossible to deal in detail with all the many points which had been raised by the critics also that such eminent authorities as Col McCarrison and Col Vedder had been unable to help in the important question of the relationship between epidemic dropsy and beri beri but he hoped that they would examine the material which had been collected by his assistants and himself and then give them the benefit of their views on the subject

He also asked for a broad consideration of the question as well as an examination of details as it was only by making a wide survey that the truth could be arrived at

The opinion expressed by Dr Heiser would be cordially agreed to by everybody, it was most desirable that the diet should be attended to in regard to its vitamin content, but he asked Dr Heiser to accept the view that the storage of rice should also be regarded as important

PROPHYLAXIS AND CURE OF BERI-BERI BY VITAMIN-PREPARATIONS.

BY

B C P. JANSEN

AND

W. F DONATH.

SINCE the researches of Eijkman and Grijns, now more than a quarter of a century ago, we know the cause of beri beri to be a deficiency of a certain substance in the food Funk gave the name vitamin to this substance, but now we prefer to call it the anti beri-beri vitamin or anti-neuritic vitamin, in distinction from other vitamins As we knew the cause, the cure was obvious to supply the unpolished rice, rich in vitamins instead of the vitamin deficient polished rice This cure was successful in every place it was applied Foremost the soldiers, the prisoners, the mining coolies in short all those who do not buy their food themselves but who get it from the government or industrial and other companies, and who formerly were particularly subject to beri beri, had a great benefit by these measures But there is another class of people, to wit the poorer inhabitants of the great cities in the rice eating countries who do not pound their own rice, but who have to buy it from the grocer, who sells only polished rice In preceding Congresses of the F E A T M there were delegates, who wanted to forbid the manufacture and trade of polished rice or would hinder it by heavy taxes But the Congress never accepted these proposals and we think they were right in rejecting them, for apart from minor objections, the unpolished rice is not an article of trade, because it is very quickly tainted But now we have isolated the anti beri beri vitamin, which appears to have a very simple structure $C_6 H_{10} ON_2$ We have good hope that another solution of the rice problem will soon be possible

For we may expect, that within a measurable space of time, the synthesis of this vitamin will be brought about, and that the synthetical vitamin will be rather cheap From experiments on birds, we know that 500 grammes of unpolished rice, that is the daily amount consumed by one man, contain about 1 milligram of anti beri beri vitamin Thus, even if the price of one kilogram of synthetically prepared vitamin should be £100, the monthly costs for one man would be less than a penny

Therefore, my opinion is that the most urgent measure for controlling beri beri is not a legislative one, but that it is the research for a cheap synthesis of the

anti beri beri vitamin However it probably will take some time before that stage will be attained

Therefore for the time being the medical laboratory at Batavia has started the sale of some preparations obtained from rice polishings We do not claim any priority for these preparations for it is well known that similar preparations have been distributed for a long time by other laboratories as in the Federated Malay States the Philippines and Japan

Three different preparations are made by the medical laboratory at Batavia

The first consists of Fuller s earth which has absorbed the vitamin from an extract of rice polishings We have brought it in the form of tablets of ¼ gramme each One tablet contains the vitamin of 7 5 grammes of rice polishings As appears from experiments on animals and also according to the experience when taken per os by beri beri patients this preparation cedes its vitamin to the organism probably in the intestine with its alkaline reaction It is not decomposed by the air a sample which we kept for nearly a year in a petri dish appeared to be as active as the fresh prepared substance If we suppose that an adult eats 500 grammes of rice daily which contains about 7 per cent pericarp he would consume the vitamin of 35 grammes of pericarp if the rice had been unpolished However in the native villages the rice is always pounded to a greater or lesser extent, a considerable part of the pericarp being lost in this way without any cases of beri beri appearing Consequently we may accept that a daily dose of 1 gramme of the above preparation corresponding to 30 grammes of bran will protect a person whose chief nourishment consists in polished rice from beri beri and that ½ gramme or 2 tablets will be sufficient for a person who with his polished rice eats a moderate quantity of other food

The second preparation is a solution made by extracting the activated Fuller's earth with baryta and by filtering and neutralizing the filtrate with sulphuric acid This extract contains in 1 c c the vitamin from ± 300 grammes of rice polishings It is especially suited for the treatment of infantile beri beri and may be mixed with cooked rice or with milk

The third kind of preparation is in the form of sterilized ampoules containing a highly purified extract of activated Fuller s earth One ampoule of 2 c cs contains 1 mg of vitamin together with ± 3 mg of other unknown substances and also some chloride of sodium to make an isotonic solution These ampoules are to be used in the acutest cases of beri beri They are so little toxic that a mouse of 20 grammes may receive a subcutaneous or intravenous injection of one ampoule, that is the dose for one man without apparent harm

The demand for these preparations which we do not yet deliver to the public, but only to physicians has greatly surpassed our expectations In the last month we delivered more than 100 000 tablets monthly

But we are well aware that however valuable these preparations may be for the cure of beri beri they are of little or no use for the prevention for hardly anybody will take every day some tablets which are considered by most people as a

medicine Therefore, we have tried to mix the preparation with table salt, in the same way as now all over the world, recently in Dutch East Indies too the goitre is being controlled by iodized salt The first experiments we did with the crude salt were disheartening within one month the vitamin was destroyed by the crude salt *But then we took pure salt and the results were much better* after two months we could not discover any destruction of the vitamin We had not yet the opportunity to examine this vitamin salt after a longer time than two months

We have laid much stress upon the necessity of preparing the tablets as cheap as possible At present we can deliver 10 tablets for a penny But *we have made many little changes in the manufacture, and we hope soon to* deliver 20 tablets for the same price Thus, a person, who takes 2 tablets a day has a daily expense of 1/10 of a penny

DISCUSSION

Prof J L Rosedale (Straits Settlements) It is a matter for sincere congratulation to the authors that they have succeeded in isolating one of the factors of B vitamin but the discussions of this section of yesterday and to-day have brought out, in dealing with beri beri that balance of diet as a whole is the important factor Each dietary essential has to be considered in connection with all the other items in dealing with the subject of beri beri in its widest sense

Dr B C P Jansen (Netherlands East Indies) Replied to Dr Rosedale that *he also thought there were two (or more) B vitamins, but that the* isolated vitamin $C_6 H_{10} ON$ was especially responsible for preventing beri beri

AN INVESTIGATION TO DETERMINE A SATISFACTORY STANDARD FOR BERI-BERI PREVENTING RICES

BY

LIEUT. COL. EDWARD B. VEDDER, MEDICAL CORPS, U. S. ARMY
U. S. Army Medical Department Research Board, Manila, P. I.

AND

R. T. FELICIANO
Chemist, Bureau of Science, Manila, P. I.

I. THE METHOD EMPLOYED

Tues. Dec. 2 to

ALTHOUGH medical authorities still differ with regard to a number of details concerning the ætiology of beri-beri, there is a very general consensus of opinion to the effect that beri-beri is a deficiency disease, produced whenever, in the absence of an adequate mixed diet, highly milled rice is used as the main food staple; and that the disease can be prevented by the substitution of a sufficiently under-milled rice. The most striking illustration of this fact with which we are familiar is the case of the Philippine Scouts. For a number of years (1902–1909) while they were supplied with the best grade of highly milled rice, beri-beri was the most important cause of admission to sick report for these native troops, often reaching as much as ten per cent of the entire number (5,000). In 1910 the substitution of under-milled rice was made. Beri-beri at once declined as a cause of admission and at the end of a year when the substitution had been made universally effective, beri-beri was completely eradicated. Since that time under-milled rice only has been furnished, and during all these years beri-beri has completely ceased to appear among these troops, although living in the midst of a population where beri-beri is very common. Similar results have been obtained in a number of civil institutions in the Philippines and in other countries.

Such experiences led several sanitary authorities to recommend legislation by the various countries most concerned which would diminish the production or importation of highly milled rice. But it was promptly realized that no such law could be administered without a satisfactory legal standard for beri-beri preventing rices.

Later observations by Schüffner and Kuenen (1) and McCarrison and Norris (2) and others have shown clearly that a number of rices containing 0 4 per cent or more may produce beri beri Schüffner and Kuenen stated that rice should contain at least 0 5 per cent P_2O_5 It is unnecessary to dwell on this point for all will agree that up to the present time no satisfactory standard has been established

The resolutions passed by the Far Eastern Association of Tropical Medicine in its 1925 meeting included statements to the effect that nothing has occurred to controvert the disappearance of beri beri when an adequate diet is used, that the governments concerned should encourage research toward developing a practical test to distinguish between rices that may cause and rices that may prevent beri beri when used as a staple of diet, and that the facts be collected which may be used in classifying rice in its different stages in the process of milling

Beri beri cannot be eradicated in the countries in which it is endemic without legislation and legislation waits on the determination of a satisfactory standard for beri beri preventing rices Therefore, when one of us was assigned to the U S Army Board for Medical Research at Manila it appeared that this was the most important problem connected with beri beri awaiting solution Work was commenced in October 1925 and was continued without interruption until October 1927

Plan of the Work

It was determined to procure a series of 200 different samples of rice grown in different localities and of all degrees of milling These rices were to be inspected to determine the percentage of the external layers of the grain still adhering to them, and were to be submitted to chemical analysis At the same time they were to be fed to pigeons to determine their actual beri beri producing potentialities The chemical analyses and the feeding experiments were to be carried on independently and the results so obtained subsequently combined The actual details of this simple plan are given fully, in order that there may be no question as to how the results were obtained

(1) *Procurement of Rice Samples*—Twenty samples were purchased in the open market These samples were all machine milled but came from widely separated localities (three were from China) and the degree of milling was by no means uniform

Twelve rices were secured from the Quartermaster of the U S Army Seven were samples of under milled rice furnished to the Philippine Scouts and five were samples of choice highly milled rice Each sample was a different purchase by the Quartermaster, from wholesale dealers

Samples 10—15 inclusive were obtained as follows —two different varieties of unhusked rice (palay) were taken to a primitive mill in which the milling of rice was carried on by water power As the water wheel revolved it lifted heavy pestles which were later released and fell into stone mortars Both of these samples of

rice were submitted to this milling action for varying periods of time The sample first removed (No 1) was under milled that removed next (No 2) more completely milled and the third and last (No 3) was highly milled white rice By consulting the tables it will be seen that both samples of rice when under milled prevented the appearance of polyneuritis when fed to pigeons and that polyneuritis occurred with both samples when highly milled

Ten samples of rice seed (palay) were sent to me from Java through the courtesy of General H M Neeb and Dr P J S Cramer Director of the Experiment Station Department of Agriculture Java These samples were hand pounded in Manila thus receiving different degrees of polishing

The remaining 152 samples were procured for me by Dr Stanton Youngberg Director of the Department of Agriculture of the Philippines These samples of many different varieties of rices were procured from various islands and provinces of the Philippines and were all hand pounded so that no two rices were precisely similar in degree of milling I wish here to express our great obligation to Dr Youngberg Without his cordial and continued co operation it would have been impossible to have obtained such a large series of different rices

Ten kilos of each sample of rice were purchased The rice was kept in tightly covered tin cans in a dry store room each can being labelled with the serial number of the rice As experience promptly showed that weevils moth and other insects developed in rice so kept a vial of chloroform with a loose stopper was buried in each sample The escaping vapour promptly killed all insects and the rices kept in this manner remained in good condition during the 100 days that the experiment lasted

(2) *Inspection for Pericarp*—One hundred grains of rice taken at random were stained with Grams iodine solution for one minute after which the iodine was rinsed off with water Each grain was then examined and the amount of pericarp remaining was expressed in per cent the whole pericarp with the rice embryo intact representing 100 per cent This method appears very rough and inaccurate yet long experience in selecting under milled rices for the Philippine Scouts had demonstrated the fact that it is possible in this way to pick out invariably a grade of rice that will prevent beri beri The results of this inspection are included in Table I

(3) *Chemical Analyses*—The antineuritic vitamin is undoubtedly chiefly contained in the embryo and the aleurone layer of the rice grain The outer layer the pericarp contains a high percentage of mineral matter and if most of the pericarp is retained the embryo is often also present The majority of the fat of the grain occurs in the aleurone layer For these reasons the chemical estimation of mineral salts (Ash) or fat might be as good an index as P_2O_5 which was chosen only because the phosphorus of the grain is also chiefly contained in these external layers None of these substances are chemically related to the antineuritic vitamin which contains no phosphorus fat or mineral salts This vitamin however is a nitrogenous compound and in the absence of any direct chemical test or

Later observations by Schuffner and Kuenen (1) and McCarrison and Norris (2) and others have shown clearly that a number of rices containing 0 4 per cent or more may produce beri beri Schüffner and Kuenen stated that rice should contain at least 0 5 per cent P_2O_5 It is unnecessary to dwell on this point for all will agree that up to the present time no satisfactory standard has been established

The resolutions passed by the Far Eastern Association of Tropical Medicine in its 1925 meeting included statements to the effect that nothing has occurred to controvert the disappearance of beri beri when an adequate diet is used, that the governments concerned should encourage research toward developing a practical test to distinguish between rices that may cause and rices that may prevent beri beri when used as a staple of diet, and that the facts be collected which may be used in classifying rice in its different stages in the process of milling

Beri beri cannot be eradicated in the countries in which it is endemic without legislation and legislation waits on the determination of a satisfactory standard for beri beri preventing rices Therefore, when one of us was assigned to the U S Army Board for Medical Research at Manila it appeared that this was the most important problem connected with beri beri awaiting solution Work was commenced in October 1925 and was continued without interruption until October 1927

Plan of the Work

It was determined to procure a series of 200 different samples of rice, grown in different localities and of all degrees of milling These rices were to be inspected to determine the percentage of the external layers of the grain still adhering to them and were to be submitted to chemical analysis At the same time they were to be fed to pigeons to determine their actual beri beri producing potentialities The chemical analyses and the feeding experiments were to be carried on independently and the results so obtained subsequently combined The actual details of this simple plan are given fully, in order that there may be no question as to how the results were obtained

(1) *Procurement of Rice Samples* —Twenty samples were purchased in the open market These samples were all machine milled, but came from widely separated localities (three were from China) and the degree of milling was by no means uniform

Twelve rices were secured from the Quartermaster of the U S Army Seven were samples of under milled rice furnished to the Philippine Scouts, and five were samples of choice highly milled rice Each sample was a different purchase by the Quartermaster, from wholesale dealers

Samples 10—15 inclusive were obtained as follows —two different varieties of unhusked rice (palay) were taken to a primitive mill in which the milling of rice was carried on by water power As the water wheel revolved it lifted heavy pestles which were later released and fell into stone mortars Both of these samples of

rice were submitted to this milling action for varying periods of time The sample first removed (No 1) was under milled, that removed next (No 2) more completely milled, and the third and last (No 3) was highly milled white rice By consulting the tables it will be seen that both samples of rice when under milled prevented the appearance of polyneuritis when fed to pigeons and that polyneuritis occurred with both samples when highly milled

Ten samples of rice seed (palay) were sent to me from Java, through the courtesy of General H M Neeb and Dr P J S Cramer, Director of the Experiment Station Department of Agriculture Java These samples were hand pounded *in Manila thus receiving different degrees of polishing*

The remaining 152 samples were procured for me by Dr Stanton Youngberg Director of the Department of Agriculture of the Philippines These samples of many different varieties of rices were procured from various islands and provinces of the Philippines and were all hand pounded so that no two rices were precisely similar in degree of milling I wish here to express our great obligation to Dr Youngberg Without his cordial and continued co operation it would have been impossible to have obtained such a large series of different rices

Ten kilos of each sample of rice were purchased The rice was kept in tightly covered tin cans in a dry store room each can being labelled with the serial number of the rice As experience promptly showed that weevils moth and other insects developed in rice so kept a vial of chloroform with a loose stopper was buried in each sample The escaping vapour promptly killed all insects, and the rices kept in this manner remained in good condition during the 100 days that the experiment lasted

(2) *Inspection for Pericarp* —One hundred grains of rice taken at random, were stained with Grams iodine solution for one minute after which the iodine was rinsed off with water Each grain was then examined, and the amount of pericarp remaining was expressed in per cent the whole pericarp with the rice embryo intact representing 100 per cent This method appears very rough and inaccurate yet long experience in selecting under milled rices for the Philippine Scouts had demonstrated the fact that it is possible in this way to pick out invariably a grade of rice that will prevent beri beri The results of this inspection are included in Table I

(3) *Chemical Analyses* —The antineuritic vitamin is undoubtedly chiefly contained in the embryo and the aleurone layer of the rice grain The outer layer the pericarp contains a high percentage of mineral matter and if most of the pericarp is retained the embryo is often also present The majority of the fat of the grain occurs in the aleurone layer For these reasons, the chemical estimation of mineral salts (Ash) or fat, might be as good an index as P_2O_5 which was chosen only because the phosphorus of the grain is also chiefly contained in these external layers None of these substances are chemically related to the antineuritic vitamin which contains no phosphorus, fat or mineral salts This vitamin, however is *a nitrogenous* compound, and in the absence of any direct chemical test or

satisfactory colour reaction for its precise quantitative estimation there was a possibility that amido nitrogen content might prove to be a good chemical index* Total nitrogen is valueless because the protein of rices varies so considerably irrespective of the vitamin content

The methods used (Feliciano) were as follows —

(1) Moisture Determined by drying a known weight of sample (5 grammes) in an electric oven at 100°C to 105°C until a constant weight was obtained The loss of weight represents the moisture present

(2) Ash was determined by properly incinerating the dried sample from the moisture determination The weight of the white or grey residue, free from carbon represents the ash content

(3) Fat Fats were determined by extracting a weighed sample of dried finely powdered rice with pure ether in a Soxhlet apparatus for 48 hours The extract was freed from ether and moisture and weighed This result was checked by drying the extracted rice and finding the loss in weight

(4) Phosphorus Pentoxide One gramme of the sample is carbonized in a porcelain dish Add 0 2—0 4 grammes magnesium nitrate Ash until white Place the dish containing the ash in a beaker of 400 c cs and add a sufficient quantity of concentrated nitric acid immediately followed by distilled water Heat until solution is obtained Neutralize the acid with ammonia then add two or three drops of concentrated nitric acid Add 25 c cs of the molybdic acid solution Stir to induce precipitation and let stand in a warm place at about 40°C overnight Filter through purified asbestos wash thoroughly with cold water until free from acid then transfer back to original beaker Add 50 c cs distilled water and 40 c cs N/10 NaOH Heat until precipitate is completely dissolved Titrate back excess NaOH with N/10 H_2SO_4 using phenolphthalein as indicator Calculate as P_2O_5 (*see* Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists page 2)

(5) Nitrogen was determined by Gunning s modification of *Kjeldahl s method*

(6) Amido nitrogen Albuminoid nitrogen is determined, and substracted from the total nitrogen to obtain the amido nitrogen Albuminoid nitrogen Place 0 7 gramme of the sample in a beaker Add 100 c cs water and heat in a steam bath for 10 minutes, add a quantity of cupric hydroxide reagent containing about 0 5 gramme of the hydroxide, stir and *filter* when *cold* wash *with cold* water and without removing precipitate from filter determine the nitrogen by Gunning s modification of the Kjeldahl method The filter paper used must be free of nitrogen (Official and Tentative Methods of analysis of the Association of Official Agricultural Chemists Chapter IX paras 9 and 10)

All results were calculated on the original weight of the rice rather than the dry weight because this is the method in general use in determining the P_2O_5

* This was originally suggested by Chamberlain Vedder and Williams (1)

content of rices submitted for routine analysis, since rice is not sold or consumed by dry weight However, the percentages by dry weight were calculated for a considerable number of the rices in the hope that this more accurate method would reduce the number of rices producing irregular results It was found that *there was no significant difference in the ultimate results whether calculations* were made on original weight or dry weight

(4) *Feeding Experiments* —Pigeons were selected for feeding because they are even more susceptible to polyneuritis than fowls and are readily handled Four pigeons were fed upon each sample of rice allowing them all that they would eat A jar of water was in each cage but no other food was given or could be obtained by the birds The birds were fed and observed daily by one of us (Vedder) and the date on which each bird first developed symptoms of polyneuritis was observed and entered in a note book The bird was watched until it became obviously paralysed often with retraction of the neck When thus on the point of death the bird was treated by administering small amounts of rice polishings (tiqui tiqui) or an extract of the same Prompt recovery after this treatment was almost invariable and confirmed the original diagnosis of polyneuritis (see Plate XVI figs 1 and 2) In cases of death without obvious symptoms of polyneuritis a post mortem examination was made to determine the cause of death and the sciatic nerves were removed and stained by the Marchi method to determine the existence of degeneration *In any case in which the results of this feeding experiment could* be considered doubtful because of loss of birds from intercurrent disease or for other reasons the experiment on that rice was repeated with a new group of birds Therefore nearly 900 birds were used

The pigeons under experiment were confined in groups of four in a series of fifty six wire cages 36" × 36" × 30" placed upon a concrete foundation and covered by a galvanized iron roof Each cage was numbered and provided with a bamboo roost and a similarly numbered wooden box to hold the rice The cages (shown in Plate XVII) were ideal for the experiment and were specially constructed by the Bureau of Science for this purpose We desire here to express our obligation to Dr William H Brown the Director of the Bureau for providing us with these cages and for facilitating the work in every possible way

In estimating the beri beri producing potentiality of a rice there are two factors to be considered —the number of individuals that develop the disease and the rapidity of development of the disease An attempt has been made to express both of these factors in a single figure called the beri beri producing factor The number of birds that developed beri beri was expressed as the per cent of the total number used in the experiment and this percentage was divided by the average number of days elapsing from the time the rice was first fed until the first symptoms of poly neuritis appeared Thus the higher the percentage of the birds that developed beri beri and the shorter the depletion period the greater does this beri beri producing factor become

Pigeons may occasionally develop polyneuritis as early as fifteen days on a very deficient rice. It was assumed that if none of the four birds developed polyneuritis after 100 days of feeding that the rice afforded sufficient protection and the experiment was discontinued. *In this connection it may be emphasized that as* pigeons are far more susceptible to polyneuritis than is the human race to beri beri it may reasonably be claimed that any rice that protects pigeons from polyneuritis for 100 days will prevent the appearance of beri beri in man, even when used as an exclusive diet, which is seldom the case.

The results of this work including the chemical analyses of each rice with the effect of feeding to fowls are presented in Table I. The figures for each chemical ingredient were also arranged in sequence from the lowest to the highest and *charted, together with the beri beri producing factor for each rice (Charts 1—8).*

TABLE I

No	Name	Locality	Percentage of Pericarp remaining	Chemical Analysis							Result of feeding	
				Moisture	Fat	P_2O_5	Ash	Total	Nitrogen	Amino Nitrogen	Beri beri Percentage	Beri beri Factor
1	B B	Nueva Ecija	10		0 72	0 35	0 61	1 72	1 27	0 18	50	0 98
2	2 Red	Pampanga	10		1 37	0 5[illegible]	0 91	2 87	1 02	0 38	0	0
3	H	Nueva Ecija	0		0 70	0 31	0 70	1 71	1 12	0	50	1 10
4	B	Tarlac	8		0 77	0 42	0 85	2 04	1 03	0	100	1 0[illegible]
5	A	Pangasinan	10		0 71	0 45	0 71	1 87	1 16	0	75	1 48
6	1 Red	Pampanga	15		0 71	0 47	0 81	2 07	1 10	0	75	1 50
7	Magalang Red	"	5		0 82	0 40	0 60	1 8[illegible]	1 05	0 55	75	1 59
8	Scout Rice	Pangasinan	35		1 84	0 73	1 13	3 70	1 11	0 43	0	0
9	Choice Rice	Pampanga	0		0 30	0 [illegible]	0 35	0 90	1 [illegible]	0 05	100	4 00
10	Apostol 1	Laguna	90		1 64	0 66	1 19	3 45	1 05	0 02	0	0

TABLE I—*contd*

	Name	Locality	Percentage of Pericarp remaining	Chemical Analysis							Result of feeding	
				Moisture	Fat	P_2O_5	Ash	Total	Nitrogen	Amido Nitrogen	Beri beri Percentage	Beri beri Factor
	Apostol 2	Laguna	24		1 03	0 53	1 04	2 60	1 09	0 01	25	0 03
	„ 3		10		0 50	0 39	0 64	1 [illegible]3	1 03	0 1[illegible]	100	3 03
	Mangasa 1		95		1 74	0 69	1 27	3 70	1 17	0 14	0	0
	„ 2		80		1 18	0 58	1 05	2 81	1 16	0 12	0	0
	„ 3		5		0 49	0 36	0 72	1 57	1 09	0 04	100	3 47
	Choice Rice	Pampanga	0		0 54	0 33	0 50	1 37	1 00	0 16	100	3 70
	„		0		0 54	0 39	0 50	1 43	1 13	0 04	100	1 75
	Scout Rice		95		1 35	0 56	0 88	2 79	1 14	0	0	0
	AA	Tarl	5		0 81	0 44	0 60	1 85	1 1	0 05	100	2 56
	AX		5		0 94	0 35	0 63	1 96	1 03	0	100	2 56
1	2 Red		90		1 76	0 55	1 08	3 39	1 14	0 1[illegible]	0	0
	Inantipolo	Rizal	55		1 07	0 55	0 82	2 44	0 98	0	0	0
3	Mamabunac		10		0 94	0 47	0 76	2 17	0 93	0 01	75	2 56
4	Binankero		10		1 04	0 46	0 75	2 25	1 [illegible]5	0 0[illegible]	100	1 85
	Scout Rice		95		2 69	0 78	1 20	4 67	1 [illegible]3	0 05	0	0
6	Choice Rice	Pampanga	8		0 57	0 43	0 65	1 65	1 04	0	100	2 27
7	Imonga	Laguna	85	10 54	1 34	0 64	1 04	3 02	1 [illegible]3	0	0	0
8	Lawlaw		95	10 98	1 95	0 97	1 36	4 28	1 59	0 05	0	0
	Mangasa		95	10 37	1 84	0 49	0 81	3 14	1 [illegible]6	0 05	0	0
0	Kinuristina		80	10 85	1 [illegible]0	0 73	1 17	3 10	1·60	0 05	0	0
1	Dinomero		98	11 13	2 03	0 86	1 46	4 35	1 18	0 06	0	0
32	Kinasasay		83	10 26	2 03	0 73	1 22	3 98	1 20	0 06	0	0
33	Sinenora		88	10 30	1 81	0 67	1 03	3 51	1 0[illegible]	0 06	0	0
34	Saigon	Nueva Ecija	80	10 37	1 60	0 40	0 61	2 61	1 12	0	0	0
35	Minalbon	„ „	77	10 89	1 16	0 52	0 74	2 42	1 26	0 05	0	0

TABLE I—*contd*

No	Name	Locality	Percentage of Pericarp remaining	Chemical Analysis					Result of Feeding	
				Moisture	Fat	P_2O_5	Ash	Total	Beri beri Percentage	Beri beri Factor
36	Guinobierno	Nueva Ecija	68	10.79	1.31	0.71	0.85	2.87	0	0
37	Penas	Pangasinan	90	9.93	1.79	0.77	1.09	3.45	0	0
38	Ubanan	,,	78	10.13	1.81	0.75	1.02	3.58	0	0
39	Minalabon	,,	80	9.71	1.43	0.70	1.01	3.13	0	0
40	Minis		88	9.31	1.49	0.76	1.17	3.42	0	0
41	Imachupal		85	9.69	1.57	0.63	1.02	3.22	0	0
42	Scout Rice, QMC	Rizal	98	10.76	1.99	0.73	1.05	3.77	0	0
43	*Choice Rice, QMC*	*Pampanga*	*5*	11.16	*0.66*	*0.49*	*0.65*	1.80	75	1.00
44	Tiniaong	Laguna	96	13.22	2.05	0.81	1.35	4.21	0	0
45	Mangasa		94	11.80	2.19	0.82	1.32	4.33	0	0
46	Kalibo		95	11.20	2.43	0.85	1.38	4.66	0	0
47	Binangbang		97	13.23	2.06	0.79	1.40	4.25	0	0
48	Binikol		90	12.34	1.84	0.79	1.28	3.91	0	0
49	Magsalit		96	11.48	2.51	0.82	1.36	4.69	0	0
50	Sinadyaya		92	12.05	1.90	0.84	1.34	4.08	0	0
51	Makan		98	11.94	2.36	0.74	1.37	4.47	0	0
52	Vinagat		98	12.18	2.13	0.84	1.38	4.35	0	0
53	Quinanda	,,	90	13.01	1.25	0.82	1.34	3.41	0	0
54	Sipot	Pangasinan	75	11.55	1.07	0.64	1.04	2.75	0	0
55	Bulastog	,,	87	11.80	1.22	0.63	0.94	2.79	0	0
56	Kalibo		82	11.55	1.17	0.64	1.10	2.91	0	0
57	Madaling Aras	,,	87	12.52	1.15	0.63	1.11	2.89	0	0
58	Scout Rice, QMC	Pampanga	97	10.79	1.78	0.72	1.10	3.60	0	0
59	Macan Lamio	Nueva Ecija	10	11.80	0.22	0.52	0.89	1.63	50	1.63
60	Guinubierno	,, ,,	15	12.02	0.92	0.33	0.64	1.89	75	1.53
61	Dinagupan	,, ,,	15	12.48	0.78	[illegible]		1.90	100	1.85

TABLE I —*contd*

No	Name	Locality	Percentage of pericarp remaining	Chemical Analysis: Moisture	Fat	P_2O_5	Ash	Total	Result of feeding: Beri beri Percentage	Beri beri Factor
62	Macan Cumpul	Nueva Ecija	50	11.74	1.37	0.60	0.96	2.93	0	0
63	Mimis		15	12.69	0.67	0.59	0.83	2.09	50	1.04
64	Minanteca		25	11.91	0.77	0.61	0.87	2.25	75	1.12
65	Macan Neming		16	11.54	0.91	0.[illegible]2	0.86	2.29	75	0.91
66	Minalabon		10	12.37	0.91	0.47	0.69	2.07	75	0.73
67	Saigon	"	25	12.14	1.20	0.57	0.80	2.57	0	0
68	Macan	Laguna	90	11.74	2.06	0.73	1.21	4.00	0	0
69	Kinanda		90	12.34	1.47	0.74	1.11	3.32	0	0
70	Magsalit		87	12.07	2.02	0.73	1.30	4.05	0	0
71	Binerto		95	11.52	2.39	0.77	1.17	4.33	0	0
72	Mangasa		90	11.97	1.84	0.76	1.26	3.96	0	0
73	Sinaguing		96	11.96	2.16	0.63	1.19	3.98	0	0
74	Guinangang		90	12.60	1.72	0.69	1.22	3.63	0	0
75	Sinan Jose		87	12.08	1.89	0.62	1.30	3.81	0	0
76	Dinagat		88	12.43	2.14	0.71	1.28	4.13	0	0
77	Sinadyaya		89	12.32	1.76	0.64	1.28	3.68	0	0
78	Sinandaang		98	11.59	1.80	0.58	1.09	3.47	0	0
79	Piniro		80	12.59	1.74	0.63	1.25	3.62	0	0
80	Kalibo		86	11.92	1.87	0.64	1.16	3.67	0	0
81	Scout Rice QMC	Pampanga	92	11.08	1.79	0.76	1.18	3.73	0	0
82	Magsanbay	Laguna	88	11.81	2.20	0.86	1.40	4.46	0	0
83	Piniro		89	12.40	2.35	0.82	1.24	4.41	0	0
84	Kalibo		85	12.94	1.87	0.76	1.33	3.96	0	0
85	Mangasa		90	12.40	2.06	0.82	1.40	4.28	0	0
86	Binirgen		91	12.03	2.03	1.79	1.38	4.20	0	0
87	Kinanba		92	11.88	1.94	0.78	1.31	4.03	0	0

TABLE I—*contd*

No	Name	Locality	Percentage of Pericarp remaining	Chemical Analysis					Result of feeding	
				Moisture	Fat	P_2O_5	Ash	Total	Beri beri Percentage	Beri beri Factor
140	Kinastano	Batangas	15	10 23	0 92	0 56	0 78	2 26	25	0 32
141	Inabaka	„	10	10 20	0 76	0 53	0 69	1 98	0	0
142	Binagat	„	12	10 32	0 84	0 54	0 94	2 32	100	1 58
143	Pinursige	„	50	9 74	1 11	0 53	0 83	2 47	0	0
144	Nagdamo	„	20	9 39	1 00	0 55	0 80	2 35	0	0
145	Kinawayan	„	40	9 94	0 99	0 58	0 89	2 46	25	0 31
146	Aguyod	„	30	9 70	1 27	0 53	0 90	2 70	0	0
147	Tampukoy	„	20	9 93	1 04	0 53	0 79	2 36	0	0
148	Kinandang Pute	„	10	9 78	0 59	0 51	0 83	1 93	75	1 34
149	„ Puti	„	40	10 07	0 96	0 63	1 07	2 66	0	0
150	Scout Rice QMC	Pampanga	88	12 52	1 36	0 76	1 07	3 19	0	0
151	Cotsian	Iloilo	33	10 62	1 15	0 54	0 67	2 36	0	0
152	Calubad	„	28	8 94	0 99	0 56	1·03	2 58	25	0 71
153	Cabonlong		35	9 21	1 12	0 57	0 98	2 61	75	1 83
154	Eniman	„	25	9 85	1 15	0 56	0 97	2 68	25	0 39
155	Macan Arabon	„	10	10 71	1 10	0 60	0 70	2 40	25	0 96
156	„ Quinatia	„	75	9 58	1 40	0 59	1 00	2 99	0	0
157	„ Kinalway	„	30	10 72	1 09	0 62	0 97	2 68	0	0
158	Tabao	„	45	9 62	1 68	0 66	0 95	3 29	0	0
159	Macan Tabao		75	11 08	1 32	0 65	1 09	3 06	0	0
160	Himpon	„	12	10 16	1 06	0 55	0 71	2 32	25	0 27
161	Magsalit	Bulacan	55	11 32	1 80	0 71	1 03	3 54	0	0
162	Macan Puti	„	65	10 40	1 84	0 54	1 16	3 54	0	0
163	„ Obando	„	45	10 75	1 83	0 68	1 04	3 55	0	0
164	„ Tago	„	40	10 72	1 49	0 65	0 89	3 03	0	0
165	„ Cumpul	„	45	11 60	1 39	0 63	0 94	2 96	0	0

TABLE I—*contd*

No	Name	Locality	Percentage of Pericarp remaining	Chemical Analysis					Result of feeding	
				Mo sture	Fat	P_2O_5	Ash	Total	Beri beri Percentage	Beri beri Factor
166	Dinumero	Bulacan	9ɔ	10 68	1 86	0 71	0 78	3 3ɔ	0	0
167	Tinacloban		35	9 95	1 50	0 6	0 95	3 07	0	0
168	Binuhangin		°0	8 8	1 40	0 54	0 ɔɔ	₋ 89	0	0
169	Sinanduyong		9ɔ	9 0	1 83	0 71	1⁻⁹4	3 78	0	0
170	Malagk tna Puti		85	8 99	2 10	0 83	1 ₋8	4 1	0	0
171	Solo	Java	90	8 76	1 54	0 58	0 74	°86	0	0
17	Rogol		9ɔ	7 99	2 10	0 65	1 09	3 84	0	0
173	S Rosaki		90	8 7	1 65	0 68	1 13	3 46	0	0
174	Semas		9ɔ	8 46	1 ɔ1	0 59	0 87	°97	0	0
175	Djalen		ɔ0	8 50	1 35	0 60	0 75	2 70	0	0
176	Songsan		90	8 17	1 6	0 5ɔ	0 87	2 68	0	0
177	Karang Serang		90		1 °6	0 68	1 07	3 01	0	0
178	Baok		9ɔ	10 6°	1 63	0 56	0 7°	°91	0	0
179	Merivimankott		90	10 6	1 59	0 64	0 88	3 11	0	0
180	Gedangan		98	10 35	1 50	0 55	0 9°	2 97	0	0
181	Murmuray	Tarlac	85	10 85	0°	0 69	0 96	3 67	0	0
182	Ubanan		80	10 05	1 92	0 59	0 93	3 44	0	0
183	Rama		60	10 6	1 34	0 63	1 01	°98	0	0
184	Bulalake	Cotobato	45	10 82	1 05	0 58	0 87	°50	75	0 37
185	Badyong		°0	10 86	0 97	0 56	0 80	2 33	0	0
186	Balasang	Ilocos Norte	65	8 67	1 47	0 53	0 86	°86	0	0
187	Curapo		80	8 00	1 59	0 69	1 16	3 44	0	0
188	Danacal		88	8 57	1 41	0 59	1 08	3 08	0	0
189	Duanai		70	8 51	1 66	0 66	1·03	3 3ɔ	0	0
190	Caraygay		8ɔ	8 57	1 47	0 59	0 88	2 94	0	0
191	Malaka		35	8 9°	1 53	0 56	0 79	°83	0	0

TABLE I—*concld*

No	Name	Locality	Percentage of Pericarp remaining	Chemical Analysis					Result of feeding	
				Moisture	Fat	P_2O_5	Ash	Total	Beri beri Percentage	Beri beri Factor
192	Maracatuday	Ilócos Norte	70	8 63	1 28	0 56	0 95	2 79	0	0
193	Malaka	" "	60	8 19	1 64	0 51	0 80	2 95	0	0
194	Matanobong	"	75	8 73	1 49	0 55	0 85	2 89	0	0
195	Matayesa	" "	80	8 65	1 67	0·60	0 92	3 19	0	0
196	Murmuray	" "	85	8 77	1 89	0 63	1 04	3 56	0	0
197	Santa Maria	" "	80	8 03	1 69	0·67	1 20	3 56	0	0
198	Glutinous Rice	1 Hongkong	0	9 94	0 34	0 27	0 49	1 10	100	4 00
199	" "	2 Shanghai	0	10 02	0 57	0 31	0 45	1 33	100	2 27
200	" "	3 Hongkong	0	9 63	0 59	0 25	0 48	1 32	100	3·03

II Discussion

(1) *The beri beri factor*—Our figures for this factor cannot be taken as an accurate quantitative index of the beri beri producing potentialities of these rices as is evidenced by the irregularities of the beri beri line in the charts *Nevertheless the method of arriving at this factor is believed to be correct*, and the inaccuracy is chiefly due to the fact that only a limited number of birds of various ages could be used in these experiments with the cage space and birds at our disposal Had it been possible to feed twenty birds of the same age on each rice this factor would have been more accurate, since the undoubted variation in individual susceptibility to polyneuritis would have exerted less influence on the result, and the beri beri line on the charts would have been much smoother

Whatever the quantitative error, it is obviously the same for all of the chemical constituents of each rice used, and therefore this factor may be employed as a reliable guide to the determination of the most satisfactory chemical index of the beri beri preventing rices By viewing the charts it will appear that no matter whether ash, P_2O_5 or fat are used as indicators, the character of the curve is the same At the lowest figures shown by the chemical analyses the incidence of polyneuritis is high, and as the chemical figures rise the line indicating the amount of polyneuritis drops An irregular zone is thus reached for each chemical index where some

rices produced polyneuritis and other rices afforded complete protection Finally with each index a figure is reached at which the beri beri factor drops to zero and remains there for every rice having that particular quantity or more Obviously that chemical index will be the best in which the irregular zone is reduced to a minimum

(2) *Amount of pericarp remaining on the grain as determined by staining and inspection* —This method is quite exact for completely polished rices having none of the external layers remaining and for rices having these external layers practically intact For rices having from 5 to 50 per cent of their external layers the method cannot be called exact and is probably subject to at least a ten per cent error That is it would probably be impossible to distinguish between rices having in one case 45 per cent and another case 50 per cent of pericarp But rices having 30 and 50 per cent can be readily distinguished and differences between 40 and 50 per cent are fairly noticeable

It is also possible that all of the vitamin is not always contained exclusively in the external layers of the rice and that most highly milled rices still contain traces of vitamin This possibility is strongly suggested by the fact that pigeons fed on a synthetic diet of corn starch 90 per cent egg albumen 8 per cent salt mixture 1 per cent and cod liver oil 1 per cent developed polyneuritis much faster than when fed on the most highly milled rices Thus in one such experiment all four pigeons developed polyneuritis in 12 14 18 18 days respectively giving a beri beri factor of 6 4 higher than the highest (4 0) obtained from the use of any rice in our series McCarrison and Norris (2) arrived at the same conclusion since washing or autoclaving of the most highly polished rices increased their capacity to produce polyneuritis If traces of vitamin occur as a rule in such highly milled rices certain exceptional samples may contain unusually large amounts sufficient to prevent the development of beri beri

Such unusual rices are undoubtedly the cause of some of the irregularities in our charts and may be responsible for the doubts that have arisen in the minds of some sanitary authorities concerning the validity of the theory that beri beri is caused by a deficiency arising from the consumption of highly milled rice

Admitting these sources of error the fact still remains that in the examination of the two hundred rices of this series by this method no rice having 50 per cent or more of the external layers of the grain produced polyneuritis when fed to pigeons (Chart 1) If this were taken as a standard it would have excluded 17 rices that afforded complete protection as follows 1 rice out of 15 having only 10 per cent 5 rices out of 17 having 20 per cent 2 rices out of 5 having 25 per cent 3 rices out of 6 having 30 per cent 2 rices out of 3 having 35 per cent 3 rices out of 4 having 40 per cent and 4 rices out of 5 having 45 per cent By consulting the charts it will be seen that this method of determining a rice that will prevent beri beri is more accurate than either ash P_2O_5 or fat used as an index This is a complete confirmation of previous experience indicating that this simple inspection of rice is the best method for selecting a rice that will afford protection

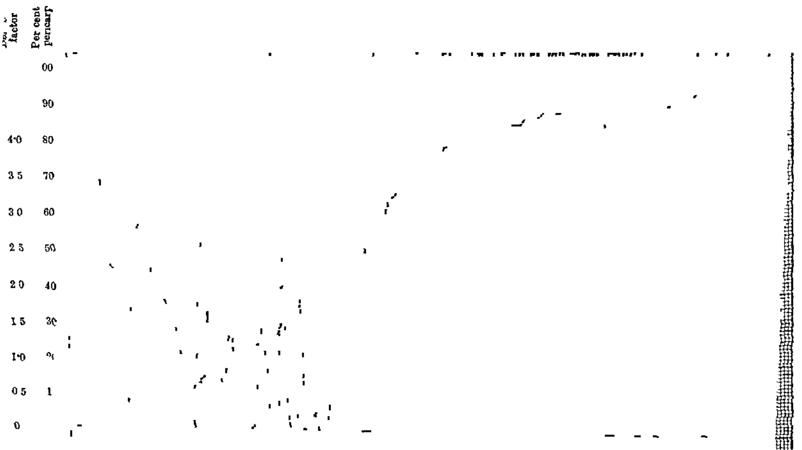


Chart 1 —Heavy line indicates per cent of the external layers of the grain remaining on each rice
Broken line indicates the beri beri factor for the same rices

Since pigeons are so much more susceptible to polyneuritis than the human race is to beri beri, it seems possible that any rice having 30 per cent of the external layers remaining on the grain will prevent human beri beri. The rarity of beri beri when hand pounded rice is eaten is therefore readily explainable * Nevertheless in purchasing rice for the Philippine Scouts an endeavour has always been made to select a rice having the external layers nearly intact. Of the seven samples used in this series only one had as low as 88 per cent of the external layers, the remaining six samples ranging from 92—98 per cent.

This undoubtedly accounts for the uniform success in the prevention of beri beri in the Philippine Scouts since 1910 when such under milled rice was furnished. This method is therefore to be recommended as the best and simplest for use in armies and institutions where some experienced and responsible official can make the examination. Unfortunately it cannot be recommended as a legal standard because the administration of a law cannot depend entirely upon the judgment of any single individual however skilled he may be.

According to the resolutions formulated by the last Congress, some terminology is desirable to designate these various degrees of milling. Accordingly it is suggested that rices in the process of milling may be stained with iodine and inspected and that those rices having from 0—20 per cent of the external layers be called *highly milled rice*, from 21—49 per cent *medium milled rice*, and from 50—100 per cent *under milled rice*. These names besides being convenient would correspond to the facts with regard to the incidence of beri beri. Beri beri may be expected to be prevalent when highly milled rice is used as a dietary staple. When medium milled rice is so used cases of beri beri may not occur at all or may sometimes occur but the cases will be more apt to be sporadic. When under milled rice is used beri beri will not occur at all.

(3) *Amido nitrogen as an index*.—The determinations for amido nitrogen appeared to bear no relation to the beri beri producing potentialities of the rices. Some of the rices having the highest amounts of amido nitrogen produced polyneuritis while a number of samples having no amido nitrogen afforded complete protection. This chemical estimation was discontinued after 50 samples were tested of which 35 were in this series. The actual figures with the results of feeding are included in Table I.

(4) *Ash as an index*.—No polyneuritis occurred on any rice having at least 1 05 per cent of ash. However, if this were taken as a standard, it would have excluded not only all the rices that produced polyneuritis but also 58 rices that afforded complete protection. The zone of irregularity covered 96 rices or almost half of the entire number (*see* Chart 2).

---

* Of course beri beri may occur when hand pounded rice is used provided the pounding is carried to the point where a highly milled rice is produced. Of the 152 samples procured through the Bureau of Agriculture which were all hand pounded 37 produced polyneuritis in pigeons.

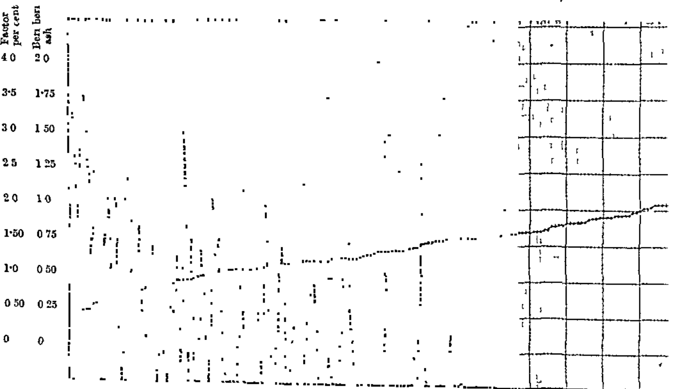


Chart 2 —Per cent of ash and beri beri factor for each rice

(5) *P_2O_5 as an index* —No polyneuritis occurred on any rice having at least 0 62 per cent P_2O_5 This standard would have excluded 45 rices that afforded complete protection The irregular zone was also very wide including 86 rices (*see* Chart 3) It will thus be seen that the P_2O_5 standard is better than the ash but is not nearly as good as the fat standard It was evident that certain highly milled and beri beri producing rices might contain a very high percentage of phos phorus 43 samples that produced polyneuritis contained 0 4 per cent or more P_2O_5 Twenty seven samples contained 0 5 per cent or more P_2O_5 and two samples contained respectively 0 60 and 0 61 Allowance must be made for the fact that pigeons known to be highly susceptible birds were used It is probable that some of these rices would have protected fowls and still more probable that they would not have caused beri beri in man Nevertheless none of them contained 50 per cent of the external layers of the grain and three of these rices Nos 26 43 and 92 containing respectively 0 43 0 49 and 0 47 P_2O_5 were the so called choice rice sold by the Quartermaster which anyone could identify at a glance as highly milled rice that would produce beri beri It is obvious that the old standard of 0 4 per cent is too low and that an acceptable standard must lie between 0 5—0 62 preferably at the higher point since we can at least assume that no rice that protects pigeons would cause beri beri in man

(6) *Fat as an index* —No polyneuritis occurred on any rice having at least 1 28 per cent of fat or more If taken as a standard this would have excluded 25 rices that afforded complete protection The zone of irregularity included 54 rices (Chart 4) It is clear therefore that the per cent of fat would constitute a better index for a beri beri preventing rice than the ash or P_2O_5

A satisfactory standard should exclude all rices that produce beri beri and should exclude none or at least very few rices that afford complete protection Neither the ash P_2O_5 or fat fulfil these requirements There remains the possibility that some combination of these three chemical determinations would be better than either one alone For when a highly milled rice contains an unusually high percentage of phosphorus it is possible that either the mineral salts or the fat may be low and thus the irregularities of any single determination would be corrected

(7) *P_2O_5 plus Ash* —The percentages of P_2O_5 and ash were added and the total so obtained charted as before No rice containing a total of 1 70 or more P_2O_5 plus ash produced polyneuritis This standard would have excluded 43 rices that protected and the zone of irregularity included 84 rices (Chart 5) This combina tion is evidently very little better than P_2O_5 alone

(8) *P_2O_5 plus Fat* —When these were totalled and charted it was found that no rice having 1 77 per cent or more of combined P_2O_5 and fat produced poly- neuritis in pigeons This standard would have excluded only 14 rices that afforded protection and the zone of irregularity included 40 rices (Chart 6) This is a distinct improvement on the fact alone and it is evident that some of the irregularities have been corrected by the use of this combined standard

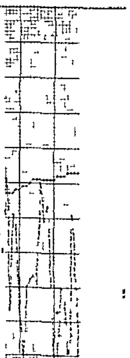


Chart 3 —Per cent of P_2O_5 and beri beri *factor for each rice*

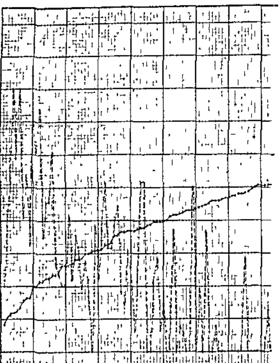
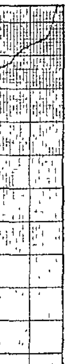



Chart 4.—Per cent of fat and beri beri factor for each rice

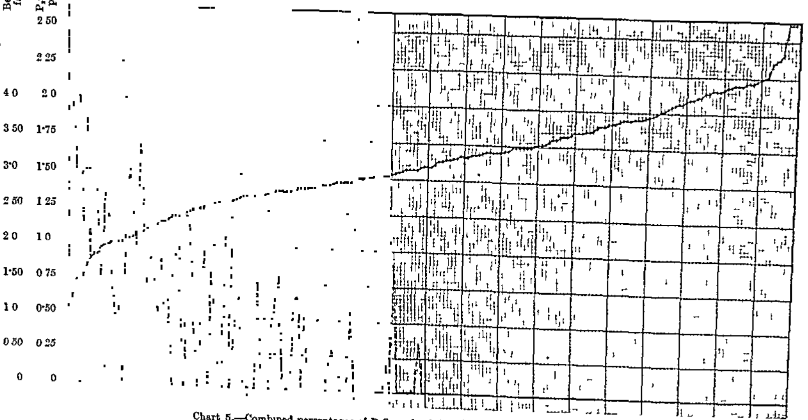


Chart 5.—Combined percentages of P_2O_5 and ash in relation to the beri beri factor

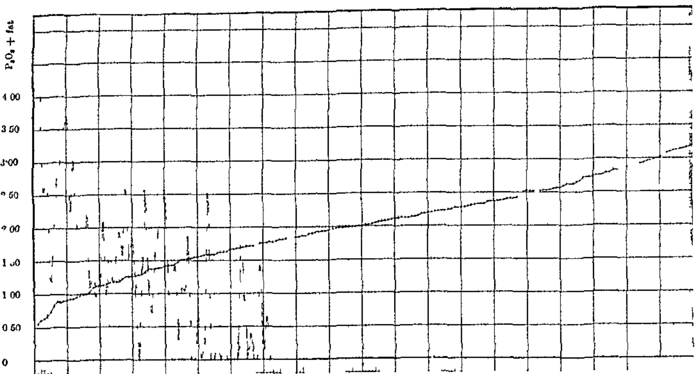


Chart 6 —Comb ned P O and fat and the beri beri factor for each rice

Percentages of comb ned P O and fat ●———●

Beri beri factor ●- - - -●

(9) *P_2O_5 plus Fat plus Ash* —The percentages of these three ingredients were added and the totals charted No rice having a total of 2 70 or more produced any polyneuritis when fed to pigeons If this were used as a standard only 13 rices that afforded complete protection would be excluded The zone of irregularity included only 39 rices (Chart 7)

(10) *Fat* (2) *plus P_2O_5*—Since the fat evidently formed the best single standard the possibility was considered that more irregularities might be corrected if the fat were given a greater weight For this purpose the percentage of fat was doubled and added to the per cent P_2O_5 No rice containing a total of 3 07 or more so figured produced polyneuritis This standard would have excluded 17 rices that protected and the zone of irregularity included 43 rices (Chart 8) This standard was evidently somewhat less accurate than the simple addition of the percentages of fat and P_2O_5

(11) *Fat* (2) *plus P_2O_5* (1) *plus Ash* (1) —The percentage of fat was doubled and added to the percentage of P_2O_5 and ash No rice having a total of 3 94 or more so computed produced polyneuritis If this were used as a standard 13 rices that afforded protection would be excluded and the zone of irregularity included 44 rices A standard derived by such a computation is obviously not as good as the simple total of P_2O_5 fat and ash which is the best standard

The selection of the most suitable index —While the total of P_2O_5 fat and ash placed at 2 70 was unquestionably the best chemical index for this series of 200 rices (excluding 13 rices that protected) it was only slightly better than the sum of the P_2O_5 and fat when placed at 1 77 (excluding 14 rices that protected) Simplicity is also worthy of consideration since the more complex is the chemical procedure the greater is the possibility of technical error and the more time consuming is the determination of the index of a given rice Moreover the ash was the poorest single index and varied more than any other constituent since it depended chiefly upon the amount of mineral salts in the soil For these reasons the standard of 1 77 P_2O_5 *plus fat would seem the more desirable*

Of the 129 rices containing this amount or more of P_2O_5 plus fat that afforded complete protection to pigeons only one (No 34) contained as little as 0 4 per cent P_2O_5 This standard may therefore be improved by adding the proviso that the amount of P_2O_5 must be at least 0 4 per cent

Using the per cent of P_2O_5 alone as a standard *all rices having at least 0 62* P_2O_5 afforded protection Of the 14 rices that afforded complete protection having less than 1 77 P_2O_5 plus fat three (Nos 157 149 and 51) had respectively 0 62 0 63 and 0 64 P_2O_5 The standard of P_2O_5 plus fat may be further improved by a proviso including any sample of *rice having at least 0 62* P_2O_5

Three of the rices that protected pigeons and having less than 1 77 P_2O_5 had at least 75 per cent of the external layers of the grain and no less than 0 5 per cent P_2O_5 (No 54 75 per cent No 35 77 per cent and No 22 85 per cent) The chemical standard might be improved by including any rice having at least 0 5 per cent P_2O_5 and not less than 75 per cent pericarp

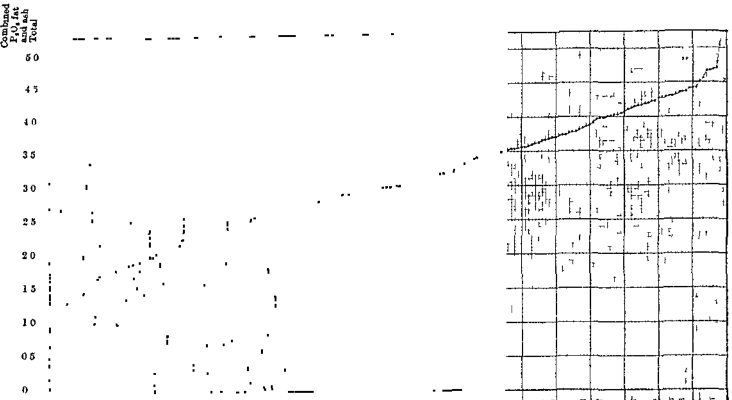
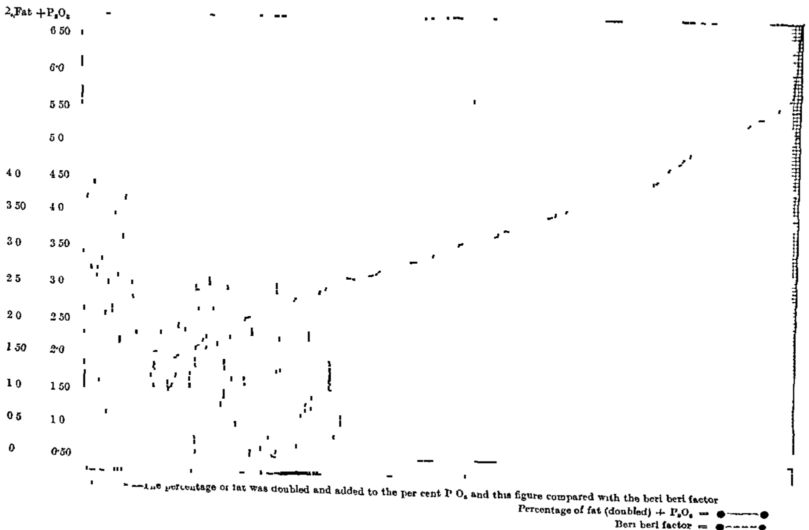


Chart 7.—Combined P_2O_5, fat and ash (total) with the beri beri factor for each rice

Percentages of combined P_2O_5, fat and ash (total) = ●——●

Beri beri factor = ●- - - -●



* —The percentage of fat was doubled and added to the per cent P_2O_5 and this figure compared with the beri beri factor

The tentative standard for beri beri preventing rice would therefore read as follows —*Any rice having 1 77 per cent of P_2O_5 plus fat but not less than 0 4 per cent P_2O_5 or any rice having not less than 0 62 P_2O_5 or any rice having not less than 0 50 per cent P_2O_5 and with at least 75 per cent of the external layers of the grain remaining*

This standard may be considered as absolutely safe for man in view of the fact that such rices afforded complete protection to pigeons when used as an exclusive diet Moreover this standard excluded only 9 rices that afforded protection out of a total of 200 or 4 5 per cent and the examination required would be little more difficult and no more time consuming than the estimation of P_2O_5 alone It is believed that this is the best chemical standard that can be devised in the absence of any direct method of estimating the exact amount of antineuritic vitamin and it is accordingly submitted as a basis for discussion by this Congress

III THE EFFECT OF DEFECTS IN THE STORAGE OF RICE AND IN ITS PREPARATION FOR FOOD ON THE PROPOSED STANDARD

The determination of a standard for beri beri preventing rice is but the first part of the solution of the administrative problem The prevention of beri beri in native populations depends not only upon the provision of an adequate beri beri preventing rice but almost equally upon the provision of facilities for the proper storage of such rice and upon correct methods of its preparation for food

In order to determine the effect of washing rice 10 samples of rice were analysed for their P_2O_5 content washed and analysed again after washing The method of washing was as follows —the rice was placed in a sauce pan and washed with running water until the water came away clear which usually required about five minutes The rice was then dried with an electric fan until all excess water was evaporated The rice was not handled or stirred during the washing The results of this experiment are shown in Table II and it will be seen that in every case the P_2O_5 content was greatly reduced Calculated on a dry basis all ten samples originally contained at least 0 4 per cent P_2O_5 while after washing no sample contained 0 4 per cent the average loss for the ten samples being 0 25 P_2O_5 Presumably the vitamin content of these rices was similarly reduced When excess water is used in cooking and strained off a further loss of P_2O_5 and vitamin occurs

Previous writers including Schuffner and Kuenen(1) and McCarrison and Norris(2) have shown that prolonged washing or soaking of the rice prior to cooking extracts and removes a considerable portion of its vitamin and that an originally beri beri preventing rice may be so converted into a beri beri producing rice This is what would be expected in view of the fact that the anti beri beri vitamin is very freely soluble in water and there would be no necessity for emphasizing this point except for the fact that ignorance of this possibility has frequently caused confusion of ideas concerning the ætiology of beri beri and has thrown suspicion upon the validity of a standard for beri beri preventing rice

TABLE II

Comparative P_2O_5 content of washed and unwashed rice

Sample Identification Numbers	Before Washing			After Washing			P_2O_5 Content
	Moisture Percentage	Calculated from sample as received	Calculated from dry basis (a)	Moisture percentage	Washed samples air dried	Calculated from dry basis (b)	Percentage difference between (a) and (b)
		P_2O_5 Percentage	P_2O_5 Percentage		P_2O_5 Percentage	P_2O_5 Percentage	
1	11.15	0.369	0.415	9.41	0.160	0.177	0.238
2	11.00	0.402	0.451	8.73	0.187	0.204	0.247
3	11.08	0.371	0.417	9.73	0.181	0.201	0.216
4	11.56	0.426	0.482	8.23	0.191	0.208	0.274
5	10.97	0.358	0.402	10.01	0.172	0.191	0.211
6	12.07	0.402	0.457	8.33	0.171	0.187	0.2[illegible]0
7	11.59	0.420	0.475	9.24	0.174	0.192	0.28
8	11.46	0.396	0.447	9.92	0.157	0.174	0.27[illegible]
9	11.91	0.414	0.470	8.07	0.172	0.187	0.283
10	11.54	0.408	0.461	9.81	0.230	0.255	0.206

At the 1912 meeting of this Association at Hongkong(3) an instance was related of two neighbouring institutions, a monastery and a convent, supplied with the same rice. There was no beri beri in the monastery, but the disease was prevalent in the convent. I was later informed by Dr Heiser that, on investigation, it was found that the monks were not very particular about washing their rice, but that the nuns, with true feminine insistence upon cleanliness washed

their rice very thoroughly and that this simple procedure was the explanation of the peculiar incidence of beri beri among the nuns

Again the idea has been advocated that beri beri is a place disease depending upon some other factor than a deficient food for its production In brief it has been found that beri beri is and has been for many years endemic in certain narrow tracts in India and that the disease occurs in these endemic areas even though hand pounded under milled or parboiled rice is used

It is by no means necessary to assume that beri beri is a place disease or that its etiology is any different in India than in the Philippines or other countries in order to explain such facts Not knowing the food habits in India we do not presume to say what they are But it can hardly be wrong to assume that the people in such endemic regions of India are like people everywhere else in being firmly addicted to some particular diet cooked in some special manner It follows that if there be any deficiency or abnormality in the diet or method of cooking that this tendency is fixed and handed down from generation to generation and that this alone will account for the fact that beri beri is endemic in certain localities It seems probable that excessive washing of rice boiling in an excessive quantity of water or some other peculiarity in its preparation is sufficient to account for the incidence of beri beri in these endemic areas among people using under milled or parboiled rice

Such erroneous food habits cannot be changed by sanitary regulations and can be changed but slowly as the result of education and therefore constitute one of the most serious difficulties in the eradication of beri beri in all countries It is to obviate such difficulties as much as possible that we have recommended such a high standard for a beri beri preventing rice A lower standard would undoubtedly suffice if it were not for such factors as excessive washing pressure cooking and other procedures that cannot be foreseen But the standard recommended at least provides a considerable margin of safety

At least the storage of rice is subject to sanitary regulation and deserves in [illegible]

[illegible] venting polyneuritis in fowls and that under milled rice might be kept for one year in a damp place and that although then musty and unfit for human consumption it still prevented the development of polyneuritis in fowls when fed as an exclusive diet On the other hand numerous apparently well authenticated instances have been related in which more or less prolonged storage of under milled rice resulted in the production of beri beri An explanation of this discrepancy is desirable

During the course of the experimental work on the determination of a standard for beri beri preventing rice it was early noted that some samples of rice were heavily infested with insects Twenty of such heavily infested samples were selected for a special series analysed and kept in cans as described previously,

except that no chloroform was added to kill the insects which were permitted to live in the rice These rices were fed to pigeons and at the end of 100 days when the experiment was discontinued the remainder of the rice was again analysed Samples of these rices were submitted to Dr W Schultze the entomologist of the Bureau of Science for identification of the insects which he found to be —

(1) Rice weevil	*Sitophilus oryzae* Linn
(2) Rice moth	*Corcyra cephalonica* Staint
(3) Rust red flour beetle	*Tribolium ferrugineum*, Fabr
(4) Granary weevil	*Sitophilus granarius* Linn

The results of this experiment showing the analysis of the rice before feeding and at the conclusion of the experiment as well as the percentage of beri beri produced in pigeons and the beri beri factor are given in Table III It will be seen that in 7 rices Nos 1 2, 9, 11 15 17 and 18 all contained originally more than 1 77 P_2O_5 plus fat which, in accordance with our other work, should have prevented polyneuritis Nevertheless polyneuritis occurred with every rice used in this series But the analysis of the rice remaining at the end of 100 days showed that in no case was the content of P_2O_5 plus fat as high as it was at first nor was it in any case as high as 1 77, the highest total being 1 67 (No 18) nor is the result changed if we take the total of P_2O_5 fat and ash Again 7 rices contained originally more than 2 70 whereas at the conclusion of the experiment the highest total was 2 34 (No 1)

As the result of the depredations of insects, an average total of 2 61 was reduced to 1 71 being an average loss for each sample of rice of 0 89 per cent P_2O_5 plus fat plus ash Nor does this indicate the full extent of the damage, for the insects eat the outer layers of the grain most readily so that those rices having the greatest proportion of the external layers as a rule suffered the greatest loss Thus sample No 1 lost 1 45, No 2 1 78, No 9, 1 08, No 10 1 24, No 11 1 85 No 15 0 90 No 17 0 86, and No 18, 0 75

From such figures it is clear that a beri beri preventing rice kept in storage under such circumstances as to be subject to the depredations of insects may frequently be converted into a beri beri producing rice, for as the insects eat away the external layers of the grain an under milled rice is in fact converted into a highly milled rice The external layers being the richest food supply are evidently preferred by insects, and this accounts for the difficulty in storing under milled rice I was informed by the Quartermaster that choice rice (highly milled) could be stored a maximum of 6 months while the under milled rice intended for the Philippine Scouts could never be stored more than three months The tendency for under milled rice to become infested with insects is the main reason for the reluctance of rice wholesalers to handle such rice and some practical method of meeting this difficulty must be forthcoming if the use of under milled rice is to be enforced

No	Name	Locality	Before Feeding: Moisture	Before Feeding: Fat	Before Feeding: P_2O_5	Before Feeding: Ash	Before Feeding: Total	Analysis after Feeding: Moisture	Analysis after Feeding: Fat	Analysis after Feeding: P_2O_5	Analysis after Feeding: Ash	Analysis after Feeding: Total	Per Beri beri centage	Beri beri Factor
1	Scout Rice	Pangasinan		1 73	0 87	1 19	3 79	11 20	1 07	0 49	0 78	2 34	75	1 32
2	Apostol	Rizal	10 07	1 36	0 51	0 95	2 82	10 75	0 19	0 35	0 51	1 04	50	0 64
3	Inadhica	"	10 05	1 10	0 44	0 81	2 35	11 49	0 64	0 45	0 74	1 83	75	0 96
4	Roxas	"	10 16	1 17	0 52	0 80	2 49	11 56	0 20	0 30	0 40	0 99	50	0 56
5	Quinalibo		9 62	1 27	0 44	0 71	2 42	11 43	0 92	0 37	0 63	1 92	100	2 43
6	Cruz		10 33	1 02	0 48	0 75	[illegible]	11 57	0 64	0 43	0 67	1 71	100	2 63
7	Magsalit	"	9 79	1 06	0 46	0 81	2 33	11 91	0 68	0 38	0 71	1 77	100	1 53
8	Connor	"	9 93	1 00	0 50	0 90	2 40	11 28	0 66	0 47	0 83	1 96	50	1 38
9	Binarbang	Laguna	10 06	1 39	0 61	1 01	2 94	11 25	0 70	0 49	0 67	1 86	25	0 52
10	Binuhangan		10 28	1 01	0 60	1 46	3 07	10 74	0 53	0 53	0 77	1 83	50	0 63
11	Guinanggang		10 48	1 66	0 63	1 13	3 42	11 00	0 53	0 44	0 60	1 57	75	0 92
12	Minantica	Nueva Ecija	10 78	1 06	0 52	0 58	2 26	10 99	0 48	0 34	0 52	1 24	100	1 61
13	Macan Cumpul	"	10 91	1 00	0 56	0 76	2 32	10 97	0 53	0 40	0 53	1 46	75	1 19
14	" Polo		10 58	1 11	0 42	0 54	2 07	10 87	0 70	0 32	0 48	1 50	75	1 41
15	Binulagsac		10 11	1 35	0 44	0 72	2 71	10 68	0 75	0 36	0 50	1 61	50	0 [illegible]3
16	Macan Aga	"	10 09	1 18	0 48	0 51	2 17	10 83	0 89	0 29	0 59	1 77	75	0 93
17	Sampaguita		10 55	1 32	0 68	0 87	2 87	10 84	0 72	0 49	0 80	2 01	50	0 64
18	Macan Nineng	" "	10 58	1 53	0 72	1 04	3 29	10 82	1 17	0 50	0 87	2 54	75	0 92
19	Macaninung	Pangasinan	9 82	1 03	0 60	0 87	2 50	10 90	0 62	0 49	0 81	1 92	25	0 38
20	Cavitena	Nueva Ecija	12 40	0 76	0 59	0 75	2 10	11 43	0 48	0 44	0 75	1 67	100	2 56

In some countries where milling facilities are ample rice should be milled only as it is required for consumption and under milled rice should not be stored by the wholesaler more than a few weeks Such a regulation will be more difficult to apply in other countries like the Philippines where the rice mills have a small capacity and accordingly the rice milled from day to day must be stored until a sufficient amount accumulates to make a shipment But in any case long storage of rice is more a matter of convenience to the dealer than a necessity The U S Army Quartermaster has found no difficulty in securing special orders of *under milled rice for the Philippine Scouts at frequent intervals thus avoiding* long storage If under milled rice were produced by law, dealers should be prevented by a suitable ordinance from keeping it in storage for long periods of time

Infestation by insects may be also reduced by the enforcement of sanitary regulations in rice mills Insects breed freely in accumulations of rice polish about such mills An ordinance to regulate rice mills in the Philippines provides that in every rice mill where labour is employed all refuse waste and sweepings shall be removed at least once a day, and that an insanitary condition shall be deemed to exist if the rice in the process of milling packing, storing or transporting is not securely protected from mould and the development *of weevils and beetles which destroy the mealy layer and if the refuse* dirt and waste products incident to the milling storing or transporting of rice are not removed daily But this excellent regulation is nowhere strictly enforced

So far as under milled rice is concerned even the strict enforcement of this regulation would not entirely prevent the subsequent development of insects in the sacked rice because the grains themselves frequently carry the eggs that have been deposited upon them in the field before the rice is brought to the mill Ottow (6) in 1915 recommended the use of carbon tetrachloride as a preservative for under milled rice stating that there was no objection to the use of this substance even on a large scale For this purpose a wide mouthed bottle or tin containing the preservative absorbed by cotton was introduced into each sack of rice This killed the insects had no influence on the taste or smell of the cooked rice and is non inflammable So far as we are aware this simple method has never been given a thorough trial in any other country It is at least worthy of further consideration

Another method that has been recommended and widely used in the United States(7) for controlling mill insects in flour mills and grain elevators is the use of heat It has been found that a temperature of from 118°F to 125°F maintained for several hours to enable the heat to penetrate all the infested parts will effectively kill all insects and insect eggs and does not injure the grain in any way The heat is obtained from steam pipes and regulated by thermometers in various parts of the building Such heat is more readily applied in the tropics than in the United States

One of the largest rice dealers in Manila has agreed to give this method a trial and is fitting up a small room that can be tightly closed and heated in which 500 sacks can be treated at one time If this experiment is successful, a further report will be made

SUMMARY AND CONCLUSIONS

(1) The proportion of the external layers remaining on a given rice may be determined with reasonable accuracy by simple inspection after staining with Grams iodine solution

(2) When the rices of this series were examined in this manner it was found that no rice having 50 per cent or more of the external layers of the grain produced polyneuritis when fed to pigeons

(3) Human beri beri can also be prevented by selecting rice in this manner which is recommended as the best and simplest method for use in armies and institutions It cannot be recommended as a legal standard

(4) This method may also be used during the milling process to determine the degree of milling since it requires only several minutes to apply it

(5) It is suggested that rices in the process of milling or as sold be classified as follows —

(a) *Highly milled rice* Having from 0—20 per cent of the external layers
(b) *Medium milled rice* Having from 21 49 per cent of the external layers
(c) *Under milled rice* Having from 50 100 per cent of the external layers

(6) The results obtained in this study indicate that amido nitrogen is useless as a chemical index 1 05 per cent ash is a poor index 0 62 per cent P_2O_5 is some what better and 1 28 per cent fat was the best single chemical index for a beri beri preventing rice

(7) The chemical index proposed for beri beri preventing rice is *Any rice having 1 77 per cent of P_2O_5 plus fat but not less than 0 4 per cent P_2O_5 or any rice having not less than 0 62 per cent P_2O_5 or any rice having not less than 0 50 per cent P_2O_5 and with at least 75 per cent of the external layers of the grain remaining*

(8) No rice of this series possessing these requirements produced polyneuritis in pigeons and this standard excluded only 9 rices out of 200 that afforded protection to pigeons

(9) Since pigeons are so much more susceptible to the deficiency of anti neuritic vitamin than is man and since man seldom lives on rice alone a standard that will protect pigeons will not only protect man but provides a factor of safety

(10) This factor of safety is necessary if beri beri is to be eradicated because defects in the storage of rice or in its preparation for food may materially reduce its vitamin content

(11) Of ten rice samples tested thorough washing reduced the P_2O_5 content from an average of 0 447 to an average of 0 197 Presumably the vitamin content was similarly reduced



FIG 1 Polyneuritis in one of the birds of this series

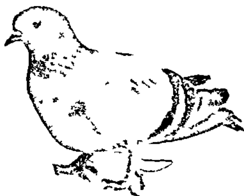


FIG 2 Same pigeon 24 hours later cured by one feeding of tiqui tiqui



EXPERIMENTAL STUDIES ON INJURIES TO PIGEONS CAUSED BY RICE OF DIFFERENT AGES AND VARIOUS MILLING PROCESSES

BY

A KESSLER M D,

Professor of Pharmacology, Tungchu University, Shanghai

THE vital part played by polished rice in the development of beri beri in countries where rice is the staple food of the population and the presence of anti beri beri vitamins in rice bran as well as in the germ, may, according to the results obtained by the research work of many scientists, be considered as well proved as the fact that beri beri is caused among other facts by a vitamin deficiency as already pointed out by Stanton before the Fifth Congress of the Far Eastern Association of Tropical Medicine It would be too great an undertaking to mention here the names and works of all the noted scientists who have played a leading part in this particular line of research work

Living in a country with a huge production and consumption of rice and learning, consequently, the various ways of treatment and its preparation, I decided, in spite of the above mentioned facts to study myself, by a thorough scientific investigation, the quality and effect of rice as a staple food upon a large number of the same class of materials, the importance of which with regard to the beri beri question has been emphasized as being of greatest consequence at every Congress of the Far Eastern Association of Tropical Medicine

The investigation by feeding, specially in cases like pigeons, being held to be a rather crude biological method I chose to take a large number of pigeons more than 150 for an extensive experiment and devote a considerable period of observation the negligence of either of which may lead to faulty conclusions

The following kinds of rice, which are used here and of which I secured sufficient quantities, were at my disposal —

(1) *New, uncured rice without the husk* —Only small quantities are milled by the peasants in their primitive mills and this rice is most used as food for fowl This rice corn is perfectly intact, only the husk is removed This rice was only a few weeks old, when it came to my hand

(2) *Uncured rice, three years old, with undamaged husk* which is used as food for birds, etc

(3) *Rice, three years old from which the husk has been removed* in the primitive mills of the peasants, consequently loses only a very small part of its original

consistency Therefore I have called it uncured ' rice Insects have eaten away a considerable part of the inner corn but the skin is only slightly damaged This rice is more expensive than the usual fresh newly ground rice and it is mainly used by well to do Chinese during the time when the new rice enters the market it is called Chen Mi ' i e old rice The Chinese consider the new rice a heavy food as compared with the old rice this latter has a bitter taste and therefore it is cooked longer and the water is thrown away Being easily digestible the old rice is recommended for invalids and expectant mothers

To avoid any inaccuracy in the experimental results and the determination of vitamin quantity in this rice it was first passed through a sieve then washed in ether to kill the insects and sieved again to make sure that no animal albumen was present in the food of the pigeons

(4) *Steamed rice six years old* which is abbreviated in Clause 4 for ' uncured rice six years old from which the husk has been removed ' This rice is called steamed old rice ' (Chen Mi ' or Nau hsin ') and is sold by the peasants to the retailers who sell it as an exceptionally light food for invalids and particularly for expectant mothers This rice is obtained by the following process —Shortly after the harvest it is gathered in big heaps covered with rice straw and according to the temperature left alone for two to six months Now and then an opening is made into the covering at the top of the heap to allow the accumulated fumes to disperse After this treatment the dry rice is milled by the peasant by which process the skin of the rice corn is to a great extent left undamaged This rice should be richer in vitamins than undermilled rice if it had not been exposed to that long heating process Unfortunately for the present it was not possible for me to control the above mentioned heating process with a thermometer which however I hope to do in the course of my further experimental studies The rice treated in this way is partly kept for several years and as pointed out above is considered a particularly light food and as such beneficial for invalids and expectant mothers In the case of this rice too the germ was partially eaten away by insects but the skin was undamaged This rice underwent the same treatment viz washing with ether etc as the other rice damaged by insects

(5) *New undermilled rice*—This rice is produced by modern rice mills which turn out big quantities of rice for distribution by the retailers This so called Sze Mi i e second class rice is mostly used by the poor population According to the information received from the retailer this rice after leaving the mill is not kept more than four months because this is the kind of rice most in demand The rice I used was only two months old when I began my experiments with it It was perfectly free from insects and undamaged

(6) *New overmilled polished rice*—Nothing in particular is to be said about this rice which the Chinese call Be Mi i e white rice It is produced in large quantities by big modern rice mills and packed in reed bags it is often stored up for one year because this rice is less used than undermilled rice The principal customers for this brand of rice are the well to do

(7) *Overmilled polished rice six years old* —This rice is known as Chen Mi ' i e old rice and is recommended as a food for expectant mothers and as a medicine Like the kind of rice mentioned before it is prepared in the bigger rice mills it is kept for years together for the above mentioned purpose Insects had eaten away the germ of the rice which I obtained and therefore this rice too was washed in ether to kill the insects and sieved after the evaporation of ether According to my information it may be assumed that the rice milled by the peasants themselves in their own comparatively primitive mills is only superficially treated the vitamin containing skin being for the greater part intact and that this rice usually is not more than one month old if it is not stored for years for the special purposes mentioned above

I used for my experiments fresh newly ground bran of both kinds sold in China viz the bran which is obtained as refuse from the under milled rice and sold as a remedy against beri beri (in my experiments called bran I) and the bran which is obtained by further milling of the under milled rice until it becomes over milled polished rice and which mixed with half per cent of calcium carbonate is used as a by food for ducks (in my experiments called bran II)

I only used fully grown healthy pigeons for my experiments which had been fed some days previously with wheat and rice The animals were housed each separately in lighted airy and spacious cages which were daily supplied with clean sand Water and rice were given in small suitable receptacles to avoid any waste of rice The amount of rice vomited by a pigeon was easily controlled on the clean sand From long lasting experiments on numerous pigeons I have found that 15 grms of rice of sufficient vitamin content per day are quite sufficient for a pigeon for its development and health I therefore gave 15 grms of rice daily instead of 20 to 30 grms as indicated by C Funk The bran was made up in pills and forcibly administered to the birds

During the experimental period the pigeons were weighed at certain intervals and any gain or loss was registered The pigeons were weighed each being wrapped up in a cloth before they got their food They were fed until the disease developed or till death The pigeons described as remained healthy have been under observation for at least four months

Corresponding to the seven different kinds of rice the experimental birds were divided into seven groups four to five pigeons in every group

Group I

The pigeons of this group were fed on 15 grms daily of the rice described under (1) The following is an example of the condition of one of these birds in the course of four months —

Initial weight 277 grms

The pigeon eats every day the whole quantity of rice of her own accord

Weight after ten days 293 grms

The pigeon always eats voluntarily her whole rice portion

Weight after another 20 days	..	.	322 grms
,, ,, ,, 20 ,,	..	.	331 ,,
,, ,, ,, 20 ,,	..	.	331 ,,
,, ,, ,, 20 ,,	..	.	324 ,,
,, ,, ,, 20 ,,	..	.	324 ,,

The pigeon remains healthy and its plumage in a good condition Increase in weight about 20 per cent of the initial weight

Group II

These pigeons received daily 15 grms of the rice described under (2) as uncured rice three years old, with undamaged husk The following is an example of the condition of one of these birds during the feeding period —

Initial weight of the pigeon .. .. .. 274 grms

During the first five days the bird has to be fed forcibly, afterwards it eats voluntarily the whole rice portion

Weight of the pigeon after 10 days	..	..	267 grms.
Weight after further 20 ,,	..	..	273 ,,
,, ,, ,, 20 ,,	..	..	280 ,,
,, ,, ,, 20 ,,	..	..	285 ,,
,, ,, ,, 20 ,,	..	..	275 ,,
,, ,, ,, 20 ,,	..	..	275 ,,
,, ,, ,, 20 ,,	..	..	273 ,,

The pigeon remains healthy and its plumage in a good condition The weight increases a little in the beginning, returns to the initial weight and remains there Whether this is due to a less efficient digestion of the rice corn owing to its hard husk remains an open question I have made the same observation with birds fed on new rice with undamaged husk

Group III

This group is subdivided into pigeons receiving —

(*a*) Daily 15 grms of the rice described under (3) as 'rice three years old from which the husk has been removed' without any by food

(*b*) Daily 15 grms of the same rice plus 1 grm of bran I

(*c*) Daily 15 grms of the same rice plus 1 grm of bran II

(*d*) Daily 15 grms of the same rice which, however, had been boiled for ten minutes

The following is the result of feeding according to scheme III*a* —

Initial weight of the pigeon . 221 grms

During the following five days the appetite increases considerably, the pigeon eating nearly the whole portion voluntarily

Weight of pigeon after ten days . .. .. 220 grms

The appetite soon decreases again and after a few days nearly all the food has to be administered forcibly

A little vomiting of rice on two days

Weight after further 20 days 236 grms

During the following four days forcible feeding exclusively a little vomiting only

During the following five days forcible feeding is continued no vomiting

On the following day distinctly pronounced beri beri of the spasmodic paralytical type according to Funk

Weight of the pigeon on this day 216 grms

Forcible feeding was continued little vomiting only

Death of the pigeon after three days

Outbreak of beri beri after 40 days Weight at the end of the experiment practically the same

Up to four weeks an increase in weight 7 per cent approximately which is gradually lost again

Life of pigeon during the experiment 43 days duration of life of the pigeons of the same group 19 to 56 days the outbreak of beri beri—partly the atrophic and partly the spasmodic form as stated by Funk—varies with the other experimental pigeons from 21 to 56 days with the exception of one pigeon which suddenly died after 19 days

The loss of weight with the exception of the above mentioned pigeon commences from the beginning and is independent of the slight vomiting but depends considerably upon the duration of the experimental period As for example the 19 days' living pigeon loses 20 per cent 26 days' living pigeon 36 per cent and 56 days living pigeon 38 per cent of its initial body weight respectively Appetite or loss of appetite was nearly the same with all the birds which can be attributed to the bad taste of rice

The influence of adding 1 grm of bran I to rice for the birds of the Group III*b* can be studied from the following results —

Initial weight of the pigeon 287 grms

The pigeon eats voluntarily the whole rice portion

Weight of the pigeon after ten days 256 grms

The pigeon still eats voluntarily the whole rice portion

Weight of the pigeon 20 days after 283 grms

After this time nearly 2 grms of rice daily was fed forcibly

Weight of the pigeon after two days more 286 grms

After this time forcible feeding of 2 to 5 grms of rice

Weight of the pigeon further 20 days after 277 grms

Now it eats the whole rice portion voluntarily

Weight of the pigeon after another 20 days 280 grms

,, ,, ,, ,, 20 280 ,,

,, ,, ,, ,, 20 ,, 283 ,,

The pigeon is healthy and its plumage in good condition It is obvious how it is accustomed itself to the taste of rice by adding bran to it which increases its appetite

The weight of the bird remains constant without taking the slight variation into consideration

The following example shows the influence of adding 1 grm bran II to rice upon the animals of the Group IIIc —

Initial weight of the pigeon	260 grms
The first two days the whole quantity of rice was fed forcibly The following days the pigeon eats voluntarily	
Weight of the pigeon after ten days	289 grms
The pigeon eats the whole rice portion willingly	
Weight after 20 days	283 grms
Pigeon eats willingly the whole rice portion	
Weight of the pigeon after 20 days	249 grm
Appetite decreases forcible after feeding with a quantity gradually increasing up to 7 grms daily is necessary	
Weight of the pigeon further 20 days after	226 grms

From this time the whole rice portion was fed forcibly every day After ten days there was a slight vomiting which continued for the next period

Weight of the pigeon after 20 days more	196 grms
The pigeon squatting with its feathers on the floor of the cage	
After ten days the bird died	
Weight of the pigeon	175 grms
The duration of life of the pigeon 100 days	
Loss of weight equals to 32 per cent of the initial weight	

About the Group III*d* i e feeding with 15 grms of rice mentioned in Clause (3) and which is to be boiled for ten minutes before administration this remains to be said that there is no difference between the boiled rice and the raw one at least so far the deliciousness of rice without the addition of bran is concerned Experiments have as yet not been made with the latter

Group II

Includes those pigeons which are to be fed with a daily dose of 15 grms from a six years old steamed and husked rice as is stated under Clause (4)

The pigeons are divided in the following way —

(*a*) Those getting only rice
(*b*) Those getting daily 1 grm of bran I in addition to rice
(*c*) Those getting daily 1 grm of bran II in addition to rice
(*d*) Those getting the mentioned rice cooked for ten minutes

The following is an example of Group IV*a* —

Initial weight of the pigeon 259 grms

Appetite changes Sometimes more sometimes less forcible feeding is necessary

Weight of the pigeon after ten days 262 grms

Appetite decreases

Weight of the pigeon after 20 days 229 grms

After ten days the whole rice portion is to be fed forcibly Slight vomiting for the first time and this continues The next day outbreak of atropic beri beri The bird shivers

Weight of the pigeon on this day 195 grms

The pigeon looks better after three days forcible feeding On the fourth day it dies

Weight of the pigeon with a small quantity of rice in its crop 202 grms

The first attack of beri beri is after 40 days Loss of weight of the bird till death equals to nearly 20 per cent of the initial weight Duration of life 44 days

The decrease in weight of the birds of this group till their extinction varies between two nearest limits The duration of life varies from 44 to 58 days Another form of beri beri except that observed in these birds could not be seen One pigeon of the remaining three died squatting its feathers which was the only sign of the disease

The influence of feeding of Group IV*b* is shown below —

Initial weight of the pigeon 278 grms

The whole rice portion is to be fed forcibly

The weight of the pigeon after ten days 298 grms

During the next 15 days further forcible after feeding of the whole rice portion Daily slight vomiting

Weight of the pigeon after 20 days from the day when it was weighed for the last time 291 grms

On the following days forcible feeding of the whole rice but no vomiting

Weight of the pigeon after ten days 314 grms.

After this weight the condition remains unchanged for eight days

On the ninth day the pigeon squats with its feathers and shivers staggers when touched and supports itself on the wings

During the following days the condition remains unchanged only the pigeon begins to vomit rice

On the next day it dies

Weight of the pigeon with empty crop 261 grms

The appearance of the symptoms of beri beri after 49 days Loss of weight is only 6 per cent of the initial weight but nearly 17 per cent of the increased weight during the first period The duration of life is 56 days

The weight of another pigeon of this group similarly decreases from the very beginning but only 8 per cent of the initial weight As to other respects the course remains practically the same during the whole experimental period

It is obvious from the following results that there is no particular change of the condition by the addition of bran II —

Initial weight of the pigeon 245 grms

The pigeon eats up voluntarily nearly the whole rice portion leaving a little behind

Weight of the pigeon after ten days 243 grms

It eats up the whole rice portion willingly

Weight after further 20 days 268 grms

It eats up the whole of rice willingly for the next eight days After this appetite decreases

Weight of the pigeon after further 20 days 250 grms

On the next day it squats with its wings stretched shivers and staggers by touch During the next six days the condition of the pigeon remains the same The whole of rice is to be fed forcibly It vomits very little rice

The *following day it expires*

Weight of the pigeon with empty crop 235 grms

Outwardly visible symptoms of disease appear after 51 days Loss of weight till extinction is practically of no importance Duration of life 57 days

The influence of rice of this group cooked for ten minutes before administration without adding bran is shown in the following experiment —

Initial weight of the pigeon 230 grms

The whole of rice must be fed forcibly

Weight of the pigeon after ten days 215 grms

Still it is to be fed forcibly

After more ten days weight of the pigeon 182 grms

On the following day the pigeon lies on the floor with scattered feathers and stretched wings

On the following day it expires

Weight of the bird with little rice in the crop *179 grms*

Appearance of the outward symptoms of beri beri after 21 days Loss of weight of the pigeon till death nearly 23 per cent of the initial weight Duration of life, 21 days

The symptoms of disease in the case of other pigeons are the same *The time* of the appearance of the outward symptoms of disease varies from 20 to 35 days the loss of weight from 18 to 25 per cent

Group I

This group is composed of three sub groups which are as follows —

(*a*) Those pigeons fed with under milled rice mentioned in Clause (3)

(*b*) Those fed with the same rice with an addition of 1 grm bran I

(*c*) Those fed with the same rice with an addition of bran II

An example of Group I*a* is as follows —

Initial weight of the pigeon 289 grms

The first six days the pigeon eats up the whole of rice voluntarily The following days the appetite decreases gradually so that the rest daily is to be fed forcibly

Weight of the pigeon after ten days 283 grms

During the following days 10 or 15 grms are to be fed daily by force

Weight of the pigeon after 20 days 270 grms

During the following days this bad appetite remains so that partial after feeding by force is necessary

Weight of the pigeon after 30 days 242 grms

The bird is still to be fed daily the whole of rice by force

Five days later the pigeon vomits for the first time Four days afterwards the pigeon squats with scattered feathers on the floor and staggers when touched during its movement

Weight of the pigeon on the tenth day from the day when it was last weighed 221 grms

The condition of the pigeon remains unchanged for seven days It allows to be fed forcibly without vomiting much rice the stool is watery and green

On the eighth day the pigeon lies dead in the cage

Weight of the pigeon with rice in its crop 198 grms

Loss of weight of the pigeon during the experiment without taking the rice in crop into consideration 35 per cent

The outward symptoms of the disease appears after 59 days not taking the loss of weight into consideration Death after 67 days

Loss of weight in near limit and duration of life of the other pigeons vary between the nearest limits of 67 One bird lost 37 per cent of its body weight and lived ten days longer

The symptoms of disease were the same with all the pigeons The loss of weight can neither be attributed to the loss of rice vomited nor to the under nourishment caused thereby as these symptoms appeared minutely during the last days and that even not regularly

The pigeons under the sub group (*b*) of Group I got daily 1 5 grm of the under milled new rice plus 1 grm bran I

The following example will show the course of feeding the pigeons with this food —

Initial weight of the pigeon . .. .. 272 grms.

Good appetite changes sometimes to a worse one so that on some days forcible after feeding is more or less necessary

Weight of the pigeon after ten days	274 grms

On the following days, appetite goes on increasing so that very little is to be fed afterwards by force

The pigeon now eats up the whole of rice willingly

Weight of the pigeon after another 20 days	280 grms
,, ,, ,, ,, 20 ,,	280 ,,
,, ,, ,, ,, 20 ,,	288 ,,
,, ,, ,, ,, 20 ,,	283 ,,
,, ,, ,, ,, 20 ,,	285 ,,
,, ,, ,, ,, 20 ,,	293

The bird is healthy and its plumage in good condition The increase of weight during the whole experimental period approximately 8 per cent

The average picture of the development of the other experimental pigeons is the same The increase in weight varies from 7 per cent to 10 per cent

Group I c Getting daily 15 grms under milled rice plus 1 grm of bran II

Initial weight of the pigeon	240 grms

The pigeon eats the whole of rice of its own accord

Weight of the pigeon after ten days	248 grms

Pigeon eats the whole of rice voluntarily during the following days

Weight of the pigeon after further 20 days	259 grms
,, ,, ,, ,, 20 ,,	275 ,,
,, ,, ,, ,, 20 ,,	274 ,,
,, ,, ,, ,, 20 ,,	269 ,,
,, ,, ,, ,, 20 ,,	265 ,,
,, ,, ,, ,, 20 ,,	270

The pigeon is healthy and its plumage in good condition Increase in weight of the pigeon during experimental period is 13 per cent approximately The weight increases for nearly seven weeks and then decreases little by little to a weight lower than the initial weight and remains there

The remaining pigeons of this group behave themselves nearly in the same way as the above mentioned type with a slight deviation in the increase of weight which lies between 6 to 10 per cent

Group II

This group together with its sub groups includes those pigeons which are fed with 15 grms of over milled, polished rice as mentioned under Clause (6) and they are as follows —

(a) Those fed with rice alone without adding anything to it
(b) Those fed with rice with an addition of 1 grm of bran I
(c) Those fed with rice with an addition of 1 grm of bran II
(d) Those fed with rice cooked for ten minutes before administration without adding anything to it

The following is an example of Group VI*a* from which the well known symptoms can be studied

Initial weight of the pigeon 246 grms

The pigeon eats up all of rice voluntarily

Weight of the pigeon after ten days 257 grms

The pigeon is *to be fed the whole of rice daily by force*

On the third day counting from the last weight taken, the pigeon vomits rice for the first time which continues till its extinction

Three days later spasmodic paralytic beri beri begins Five days later, it expired

Weight of the pigeon 213 grms

Outbreak of beri beri after 16 days Duration of life during the experimental periods is 21 days Loss of weight 12 per cent approximately of the initial weight

Other kinds of beri beri in *birds* have been observed in the case of remaining pigeons of this group The time of outbreak of the disease varies amongst the pigeons from 21 to 33 days the number of deaths being between 15 to 30 per cent *A bird of this group died already after 17 days There were no vomiting, no loss* of weight and no symptoms of beri beri

The pigeons of Group VI*b* which are fed with rice with an addition of 1 grm of rice bran I remain healthy with a slight increase in weight as can be seen *from the following example* —

Initial weight of the pigeon 284 grms

During the first ten days the good appetite changes itself with a worse one and conversely consequently for some days forcible after feeding is necessary

Weight of the pigeon after ten days 291 grms

During the following period appetite increases so that only a small quantity of rice is to be fed by force

Weight of the pigeon after further	20 days	300 grms
, ,, ,,	20 ,	308 ,
, , ,,	20	302 ,

The pigeon now eats every day all its rice portion voluntarily

Weight of the pigeon after further	20 days	302 grms
,, , ,	20 ,	305 ,,
,, , ,	20	308 ,,

The pigeon is healthy and its plumage in good condition Increase in weight, approximately 9 per cent of the initial weight

The influence of the addition of 1 grm bran II as in Group VI*c* can be seen from the following example —

Initial weight of the pigeon 240 grms

The bird eats daily its whole rice portion voluntarily

Weight of the pigeon after ten days 244 grms

The pigeon is to be fed completely by force

The forcible feeding still necessary small quantity of rice being vomited

Weight of the pigeon after further 20 days 228 grms

During the following days decrease and increase of appetite interchanges so that after feeding of a quantity up to 5 grms daily is necessary, for some days there is little vomiting

Weight of the pigeon after further 20 days . 232 grms

From this time the whole of rice is to be fed by force, very little of rice is vomited

After ten days the pigeon squats on the floor with scattered feathers and when touched staggers and supports herself on wings

Seven days later death of the pigeon, weight of the pigeon 188 grms, symptoms of beri beri after 80 days, duration of life during the experimental period 87 days loss of weight approximately 22 per cent

Spasmodic beri beri is observed only in one pigeon out of the rest Duration of life varies within small limits loss of weight between 6 to 22 per cent In the case of one pigeon increase in weight of 11 per cent is observed during the first half experimental period

Experiments with Group VI*d* give the same results as those with Group VI*a* as it is also expected from this submitted literature

Group VII

Group VII and its sub groups give us practically the same pictures as Group VI and *its sub groups respectively*

SUMMARY

Let us now sum up the important results from each of the groups in order to have a better outlook

The pigeons of Groups I (new, uncured and husked rice) and II (three years' old rice under *milled and still in its husk) remain healthy*

The pigeons of Group III (three years' old rice husked) IV (six years' old, steamed rice) V (new under milled i e, the skin is partially removed) VI (new *full milled polished rice) and VII (six years' old over milled and polished rice)* fall ill and die within 70 days if they do not get any bran

While feeding the pigeons of Group III a loss of weight is observed which is independent *of the length of time The quantity of food eaten by the pigeons* is practically the same as that in Groups I and II which remain healthy The duration of life of the pigeons varies from 19 to 56 days Eighty per cent of the pigeons show *the symptoms of atropic, spasmodic beri beri*

In the case of feeding the pigeons of Group IV there is also distinct loss of weight certainly not so much as in Group III which is also independent of the quantity *of food and length of time Duration of life between 44 to 58 days* If lying with scattered feathers be taken for granted as a sign of atropic beri beri then all the pigeons were attacked with atropic beri beri only

The pigeons of Group V suffer a loss of weight up to 35 per cent although fed with practically the same quantity of food Duration of life between 60 to 70 days Also in this group no other kind of beri beri except the atropic beri beri is observed

Group VI has nothing new in it except confirming the long known fact

Group VII has the same results as Group VI

An addition of bran I prevents the outbreak of the disease and consequently the extinction of the pigeons of Groups III V VI and VII The pigeons of Groups V VI and VII have an increase in weight of 10 per cent and those of Group III suffer no loss Administration of bran I to the pigeons of Group IV has checked the loss of weight but not the outbreak of the disease, nor has it lengthened the duration of life

Addition of bran II prevents the outbreak of the disease and consequently death too in the case of pigeons of Group V but not those of Groups III IV VI and VII

By adding bran II to the under milled and new rice of Group V the birds remain quite healthy and have an increase in weight Bran II has in this case *the same influence as bran I*

The influence of adding bran II to rice in Group III is shown below

Loss of weight is practically the same

Duration of life is increased by double

The influence of adding bran II to rice of Group IV is shown below

Loss of weight is checked and is practically zero No influence on the duration of life

The influence of adding bran II to rice of Groups VI and VII is as follows —

Loss of weight is little lowered

Duration of life is doubled

Boiling of rice so far as feeding without any addition of bran is concerned has no influence on the birds The experiments about the feeding of the birds by each sort of rice of different groups being boiled before administration and with an addition of bran are still to be made

Experiments are made with a few birds to the following respects —

Whether and how long a quantity of bran I amounting to 1 mg of Rohoryzanins Suzuki when given all at time will influence an already started disease How long this additional bran if given with the daily food after the outbreak of the disease will continue its influence upon the latter ?

The results are as follows —

(1) Over milled new and six years old rice with an additional quantity of bran for a day only shows a curing effect for six to seven days But the condition of the bird becomes again all on a sudden, worse in spite of the further addition of bran

(2) In case of three years old husked rice an addition of such a dose all for once shows a slight improvement lasting for a day only

(3) A daily addition of bran I to over milled, new rice and over milled six years' old rice has a curing effect on the bird for a long time while this daily addition to three years' old, husked rice improves the bird a little for eight to ten days and it expires

Calcium, in truth, so far the old and heated rice is not concerned, seems to have some influence, on avitaminose through ill treatment of rice Perhaps, the anti poisoning property of calcium or the influence of calcium on the inner secretion plays a part in this case My experiments cannot give any definite information to this respect

Conclusions

From my experiments I come to this conclusion that the under milled rice, if it is not brand new, also loses its vitamin contents already after some months as is already stated in Otto's experiments cited by De Langen Heat and storing up for a long time destroy the vitamin content of rice devoid of usual coating, *as we see by the husked three years' old and steamed six years' old rice* The insects have damaged rice to a great extent Rice will remain with its full vitamin content for three years if it is kept unhusked in a place not very hot, other circumstances remaining constant

This justifies the desirability of adopting the means of allowing only unhusked rice for export and import or for any sort of transport specially in those countries where it forms the principal nourishing food, as was already placed before the last Congress by De Langen

REPORT OF THE COMMITTEE ON BERI-BERI OF THE PHILIPPINE ISLANDS

I INTRODUCTION

FOLLOWING the recommendation of the Far Eastern Association of Tropical Medicine in its resolution approved at its last Congress held at Tokio, a committee to continue the investigation on beri beri has been appointed by the Honourable, the Secretary of Public Instruction An appropriation of five thousand one hundred (P. 5,100) pesos has been approved for investigation and propaganda work, the International Health Board contributing with one hundred (P 100) pesos

The Committee was appointed on the 18th October, 1926, and held eight meetings up to the present time for the discussions of the different aspects of the problem

The Committee is composed of the following —Dr Fernando Calderon Colonel Edward B Vedder, Major A Parker Hitchens, Dr Luis Guerrero Dr Liborio Gomez, Dr José Fabella, Mr A H Wells, Dr Isabelo Concepcion, Dr José Albert and Professor F O Santos, members, and Dr L Lopez Rizal, chairman

II PRESENT SITUATION OF BERI BERI IN THE ISLANDS

No change has been noted in the situation of beri beri in the islands since the last report was submitted two years ago, although to a slight degree, the same tendency to increase in the provinces and to decrease in the city has been noted for the last two years as shown in the following Table —

Mortality from Beri beri in the Philippines

Year	Manila	Provinces *	TOTAL
1910	1 441	4,128	5 569
1911	1,331	4 367	5 698
1912	1,056	4 372	5 428
1913	696	3 194	3 890
1914	839	4 102	4 940
1915	872	4 336	5 208
1916	684	5 874	6 558
1917	490	7 463	7 953
1918	731	11 866	12,597
1919	406	11 981	12,387
1920	555	12 481	13 036
1921	705	15 311	16 016
1922	648	16 241	16 889
1923	698	17 417	18 115
1924	600	18 331	18 931
1925	587	17,944	18 531
1926	526	18,678	19 204

* Including deaths registered in Manila among non residents.

No great variation has been noted as regards the distribution of the disease in the provinces compared with that reported previously, while in some provinces there was an increase, a decrease could be shown in others An analysis of the facts that might have contributed to this phenomenon failed to show any other important factor than that errors may have possibly been made in the diagnosis, knowing that the death certificates and the diagnosis of causes of death stated therein are usually prepared by laymen

Judging from the death returns, beri beri is the third in the list of the more important causes of death in the Philippines and contributes to our general mortality in about 8 per cent of the total mortality Ninety one per cent of the total deaths from beri beri occurs as infantile beri beri (Deaths among infants under one year)

There are annually an average of 16 500 deaths in round numbers ascribed to infantile beri beri which represents 28 10 per cent of the total deaths under one year of age and 43 21 per thousand births

Beri beri prevails during the months of October, November, December and January

The disease is widely distributed in the Philippines, showing however a great variation in the range of mortality in the different provinces according to the mortality statistics compiled The provinces of Central Luzon such as Cavite Nueva Ecija Bataan Rizal, Laguna Batengas Tarlac, Bulacan and the Islands of Mindoro and Marinduque contribute with the highest rates of mortality (from 20 to 51 per 10 000 population)

III Rice

It is still generally admitted that where rice forms the staple of diet, beri beri prevails

At the last meeting of the Far Eastern Association of Tropical Medicine (Tokio 1925) resolutions were approved to the effect that the governments concerned should encourage research towards developing a practical test to distinguish rices that may cause or prevent beri beri, and that facts be collected which may be used in classifying rice in its different stages in the process of milling The Committee is fortunate in having amongst its members Colonel Edward B Vedder, Chairman of the United States Army Medical Research Board in the Philippines well known for his previous works and investigations on beri beri in the islands who has willingly undertaken the task of performing the investigation of this aspect of the problem After about two years' work, he submitted a lengthy report of which, for the sake of brevity, only parts will be quoted throughout this report

Importation and production of rice in the Philippines —From tables prepared by the previous Beri Beri Committee, supplemented by data furnished by the Bureaus of Agriculture and Customs we have been able to compile the amount

of rice imported and produced in the Philippines. The Table below shows in kilograms the amount of importation and production of rice —

Importation and Production of rice in the Philippines

Year	Total rice in kilograms	Total rice produced	Total rice imported	Percentage
1910	734 373 039	537 046 819	197 3[illegible]6 220	26 87
1911	768 306 581	584 631 873	183 674 708	23 91
1912	637 046 764	330 989 488	301 057 276	47 63
1913	784 630 153	697 649 508	86 989 555	11 09
1914	744 393 683	647 479 186	96 9[illegible]1 497	13 02
1915	725 855 541	507 413 996	218 441 545	30 09
1916	784 266 803	594 431 226	189 835 577	24 21
1917	949 567 722	802 582 007	146 985 715	15 48
1918	1 203 060 6[illegible]5	1 019 3[illegible]0 124	183 731 531	15 27
1919	1 01[illegible] 81[illegible] 736	961 093 978	50 818 758	5 02
1920	1 126 731 7[illegible]2	1 049 397 3[illegible]0	77 331 352	6 86
1921	1 256 176 224	1 197 658 507	58 517 717	4 66
1922	1 [illegible]79 237 709	1 236 947 841	42 [illegible]04 868	3 31
1923	1 339 297 905	1 272 843 866	66 449 039	5 36
1924	1 720 333 893	1 569 2[illegible] 100	151 108 793	8 78
1925	1 874 509 9[illegible]3	1 723 311 006	101 198 917	5 55
1926	1 874 099 814	1 813 615 894	70 483 920	3 77

Our production of rice is steadily increasing. Notwithstanding this fact the importation which ought to have decreased had during the last three years relatively increased in proportion to the production. However comparison of beri beri mortality and increased rice importation does not show any noticeable correlation.

Varieties of rice and rice mills —In the investigations performed by the previous Committee the correlation of the different varieties of rice and the presence of modern rice mills in each particular locality has been studied. The conclusion arrived at from the studies made, was that no correlation exists between the number and presence of rice mills in any locality and that no significant correlation is there between the different varieties of rice and between beri beri mortality taken from the death returns. If any correlation was noted it was due to the degree of polishing the proportion of P_2O_5 content the degree of unpolishing etc rather than the difference in variety.

Standardization of rice—This part of the work of the Committee has been totally undertaken by the member of the Committee Colonel Vedder with the co operation of Mr R T Feliciano chemist of the Bureau of Science

In the Philippines for a good proportion of rice a 0 5 per cent P_2O_5 content may probably be regarded as a fair standard for rice' The above was a statement copied from the report of the previous Committee on beri beri It is realized that the standard as it was found if it has any significance is only local and perhaps not applicable to other countries It is further known to all the difficulties of applying this standard as it is not always dependable due to the practice of some rice dealers in the Philippines to mix rice polishing with the sample submitted for examination thus increasing to some extent the P_2O_5 content

Degree of unpolishing—As an indirect method in determining the vitamin content of rice and seeing whether this factor (degree of unpolishing) may be taken as an index for the standardization of rice the previous Committee has (by the microscopic method) determined the degree of unpolishing (the method described in a previous report) After determining the degree of unpolishing (portion of pericarp left after milling) it was found out that the results do not show exact parallelism with the P_2O_5 content and because the Committee did not have proper facilities to actually determine the correlation between this factor and beri beri it was recommended that the investigations on the standardization of rice be continued

Fortunately for the present Committee at the time of its creation Colonel Vedder of his own accord as Chairman of the U S Army Medical Research Board had already started to work on this aspect of the problem the results of which were made available for the preparation of this report

Two hundred different samples of rice grown in different localities and of all degrees of milling were subjected to a series of studies by—first determining the percentage of the external layer of the grain still adhering to them (degree of polishing) secondly examining them chemically and thirdly determining their beri beri producing potentialities by actual feeding to pigeons

To determine the percentage of the external layer left in the grain instead of using the microscopic method employed by the last Committee Gram s iodine staining method was used *One significant fact noted from the results obtained* is that out of 200 samples 7 showed 0 per cent of pericarp remaining and these were among the choice and over milled rices from Pampanga(3) Nueva Ecija(1) and Hongkong (3 glutinous which is not commonly used except for cakes sweet meats etc) and that when native rice is found pounded or under milled a large proportion of them contain not less than 75 per cent of pericarp remaining These results will be further discussed in connection with its relation to beri beri It should be taken into consideration that the method cannot be taken as an exact measure of the remaining pericarp for rices having less than 50 per cent of their external layers An error of at least 10 per cent should be taken into account

however, for rices with the external layer practically intact and for those completely deprived of it, more accurate results are obtained

Percentage of pericarp remaining	Number of samples	Percentage of pericarp remaining	Number of samples
0	7	51—55	2
0—5	8	56—60	3
6—10	20	61—65	2
11—15	9	66—70	4
16—20	9	71—75	7
21—25	7	76—80	13
26—30	5	81—85	11
31—35	4	86—90	40
36—40	5	91—95	22
41—45	5	96—100	11
46—50	6		

Chemical analysis—Chemical analysis of the total 200 samples were made for the determination of moisture, fat, P_2O_5 ash, nitrogen and amido nitrogen

All results were calculated on the original weight of the rice rather than the dry weight, because this is the method in general use in determining the P_2O_5 content of rices submitted for routine analysis, since rice is not sold or consumed by dry weight However the percentages by dry weight were calculated for a considerable number of the rices in the hope that this method which is more accurate would reduce the number of rices producing irregular results It was found that there was no significant difference in the ultimate results whether calculations were made on original weight or dry weight

Feeding experiments—To determine the beri beri producing potentiality of the different varieties of rices under various degrees of milling, feeding experiments in pigeons were performed (about 900 pigeons were used) Pigeons were selected for feeding because they are even more susceptible to polyneuritis than fowls, and are readily handled Four pigeons were fed upon each sample of rice allowing them all that they would eat No other food was given, except water, which is provided abundantly in each cage The pigeons were observed every day and the date of the first symptoms of polyneuritis as well as other subsequent paralysis, are carefully noted down and recorded When the birds were on the point of death, they were treated by administering small amounts of rice polishings (tiki tiki) or an extract of the same Prompt recovery almost invariably followed, which thus

confirmed the previous diagnosis of polyneuritis When death occurred in cases of doubtful diagnosis autopsies were made to determine the cause of death making a careful examination of the sciatic nerves for the existence of nervous degeneration In any case in which the results of the feeding experiment could be considered doubtful because of loss of birds from intercurrent disease or for other reasons the experiments on that rice were repeated with a new group of birds '

Beri beri producing factor —Colonel Vedder has worked out a co efficient that may express the beri beri producing power of a given rice which he called ' beri beri producing factor In estimating this co efficient two factors were considered viz the number of individuals (pigeons) that develop the disease and the rapidity of development of the disease The percentage of the former to the total number of pigeons used in the experiment divided by the average number of days elapsing from the time the rice was first fed until the first symptoms of polyneuritis appeared will represent the co efficient thus the higher the percentage of the birds that develop polyneuritis and the shorter the depletion period the greater the co efficient will be

The first symptoms of polyneuritis occasionally appeared as early as fifteen days after feeding In cases that none of the birds developed the disease after 100 days of feeding it was assumed that the rice afforded sufficient protection and the experiment was discontinued Since the pigeons are more susceptible to polyneuritis than men it may reasonably be claimed that any rice that protects pigeons for 100 days will prevent the appearance of beri beri in man even when used as an exclusive diet which is seldom the case '

Results of investigation and staining of remaining pericarp —Out of the 200 samples of rice examined 115 or 57 50 per cent of the total showed a percentage of over 50 remaining pericarp while 85 or 12 50 per cent showed 50 or less than 50 per cent pericarp remaining In comparing these percentages obtained with the beri beri produced and the beri beri factor it is shown that no rice having 50 per cent or more pericarp remaining produced polyneuritis in pigeons at the same time it may be noted that 17 other samples having less than 50 per cent external layers of the grain protected against the disease as follows —1 sample of rice out of 15 having only 10 per cent 5 rices out of 17 having 20 per cent 2 rices out of 5 having 25 per cent 3 rices out of 5 having 30 per cent , 2 rices out of 3 having 35 per cent 3 rices out of 4 having 40 per cent and 1 rices out of 5 having 45 per cent As an index to show whether a rice is beri beri producing or beri beri preventing the percentage of pericarp remaining is comparatively a better one than either ash fat or $P\ O_5$

On the other hand the experiments performed seemed to suggest the possibility that all the vitamin content is not always exclusively contained in the external layers of the rice and that the most highly milled contain traces of vitamin because of the fact that pigeons fed on a synthetic diet composed of corn starch 90 per cent egg albumen 8 per cent salt mixture 1 per cent and cod liver oil

1 per cent, developed polyneuritis much faster than when fed on the most highly milled rice

Only under milled rice was used in the diet of the Philippine Scouts since 1910 followed by the complete disappearance of beri beri from the sick list among them Seven samples used in this series of 200 examinations were secured from rices furnished to the Philippine Scouts Out of these seven samples only one had as low as 88 per cent pericarp and the remaining six samples ranged from 92 to 98 per cent The remarkable success in the prevention of beri beri among the scouts was undoubtedly due to the method used in selecting rice for their diet This method is therefore to be recommended as the best and simplest one for use in armies and institutions although unfortunately it cannot be recommended as a legal standard for the obvious reason that the individual factor cannot entirely be eliminated in the appreciation and grading of rice samples submitted

Although 50 per cent of the pericarp in any of the 200 samples proved to be a protection against polyneuritis in pigeons which are comparatively more susceptible to the disease than man we feel that it needs to be determined further whether lower than 50 per cent remaining pericarp in the rice grain would afford practical protection in man taking into consideration that the method of preparation and cooking of rice among the natives in the islands reduces to a certain degree the P_2O_5 which is chiefly contained in the external layers

Ash as an index —The findings showed that no polyneuritis occurred with any rice having at least 1 05 per cent of ash If it is true that this percentage excludes all rices producing polyneuritis it also excludes 59 rices or 29 5 per cent of the samples that afforded complete protection, as follows —

0·61—1	0·82—2	0·93—1
0·67—1	0·83—2	0·94—3
0·69—1	0·85—2	0·95—4
0·72—1	0·86—3	0·96—2
0·74—2	0·87—3	0·97—1
0·75—1	0·88—3	1·00—1
0·78—1	0·89—1	1·01—2
0·79—2	0·90—1	1·02—3
0·80—4	0·91—1	1·03—3
0·81—1	0·92—2	1·04—4

As an index the ash is therefore less acceptable than the percentage of remaining pericarp and it is further to be found out whether the percenatge of 1 05 is the safest limit which would apply to rices grown in other countries Among the 200 samples submitted to chemical examination, 10 samples were received from

Java and *hand pounded* in Manila. Out of these 10 samples only 3 showed 1.05 per cent of ash or over while 7, or 70 per cent gave ash percentage ranging from 0.67 to 0.92. Notwithstanding this fact all the samples proved to be beri beri preventing rices. The relation between the beri beri factor and the percentage of ash is given in the following Table —

Table showing the relation between the Beri beri Factor and the Percentage of Ash

Percentage of Ash	Beri beri Factor									Total
	0	0.01—0.50	0.51—1.00	1.01—1.50	1.51—2.00	2.01—2.50	2.51—3.00	3.01—3.50	3.51—4.00	
0—0.24										
0.25—0.49	1					1		1	2	5
0.50—0.74	5	1	5	3	7	3	5	2	1	32
0.75—0.99	41	4	1	10	6	1	1			64
1.00—1.24	57	1	1							59
1.25—1.49	37									37
1.50—1.99	3									3
Total	144	6	7	13	13	5	6	3	3	200

Phosphorus pentoxide.—The P_2O_5 standard is better than the ash but is not nearly as good as the fat standard. Out of 200 samples examined for P_2O_5 content 21 or 10.5 per cent was found to contain lower than the old 0.46 proposed standard for beri beri preventing rice and 179 or 89.5 per cent had the limit (0.45) or more. In comparing these findings with the results of feeding experiments it was found out that the old standard 0.4 per cent is too low to be safe. Pigeons fed on rices having a minimum of 0.62 per cent of P_2O_5 did not develop polyneuritis.

A total of 99 samples of rice were found to have at least 0.62 per cent P_2O and afforded complete protection. At the same time there were 45 others that coming below this minimum afforded just the same protection. On the other hand other rices having similar or relatively a higher percentage of P_2O_5 than the

old standard, did not protect from polyneuritis, as is shown in the following Table —

Samples of rice producing polyneuritis in pigeons

Percentage of P_2O_5	0·4—0 49	0 50—0 59	0 60—61
Number of samples	43	27	2

It must not be forgotten that these experiments were made on pigeons, which are more susceptible to polyneuritis than man It is probable that certain rices with a high P_2O_5 percentage, that have not protected pigeons, would have protected man It is a fact, however, that none of these rices contained 50 per cent of the external layers of the grain To show the relation between beri beri and the percentage of P_2O_5, the following Table has been prepared —

Table showing the relation between the Beri beri Factor and the Percentage of P_2O_5

P_2O_5 per cent	Beri beri Factor									Total
	0	0 01—0 50	0·51—1 00	1 01—1 50	1 51—2 00	2 01—2 50	2 51—3 00	3 01—3 50	3 51—4 00	
0·20—0 40	1		1	1	4		3	3	3	16
0 41—0 60	43	6	6	11	9	5	3			83
0·61—0 80	73			1						74
0 81—1 00	25									25
1·01—1 20										
1 21—1 40										
1 41—1 60	1									1
1 61—1 80	1									1
Total	144	6	7	13	13	5	6	3	3	200

Results of examination of fat in rices —The results of examination of fat in 200 samples chemically examined, showed a wide variation the figures ranging from 0 22 to 2 86 as maximum Pigeons fed on rices having at least 1 28 per cent

Java and *hand pounded* in Manila Out of these 10 samples, only 3 showed 1.05 per cent of ash or over while 7, or 70 per cent gave ash percentage ranging from 0.67 to 0.92 Notwithstanding this fact all the samples proved to be beri beri preventing rices The relation between the beri beri factor and the percentage of ash is given in the following Table —

Table showing the relation between the Beri beri Factor and the Percentage of Ash

Percentage of Ash	Beri beri Factor									Total
	0	0.01—0.50	0.51—1.00	1.01—1.50	1.51—2.00	2.01—2.50	2.51—3.00	3.01—3.50	3.51—4.00	
0—0.24										
0.25—0.49	1					1		1	2	5
0.50—0.74	5	1	5	3	7	3	5	2	1	32
0.75—0.99	41	4	1	10	6	1	1			64
1.00—1.24	57	1	1							59
1.25—1.49	37									37
1.50—1.99	3									3
Total	144	6	7	13	13	5	6	3	3	200

Phosphorus pentoxide —The P_2O_5 standard is better than the ash but is not nearly as good *as the fat standard Out of 200 samples examined for P_2O_5 content* 21 or 10.5 per cent was found to contain lower than the old 0.45 proposed standard for beri beri preventing rice and 179 or 89.5 per cent had the limit (0.45) or more In comparing these findings with the results of feeding experiments it was found out *that the old standard 0.4 per cent is too low to be safe* Pigeons fed on rices having a minimum of 0.62 per cent of P_2O_5 did not develop polyneuritis

A total of 99 samples of rice were found to have at least 0.62 per cent P_2O_5 and afforded complete protection At the same time there were *45 others that coming* below this minimum afforded just the same protection On the other hand, other rices having similar or relatively a higher percentage of P_2O_5 than the

old standard, did not protect from polyneuritis, as is shown in the following Table —

Samples of rice producing polyneuritis in pigeons

Percentage of P_2O_5	0 4—0 49	0 50—0 59	0 60—61
Number of samples	43	27	2

It must not be forgotten that these experiments were made on pigeons, which are more susceptible to polyneuritis than man It is probable that certain rices with a high P_2O_5 percentage that have not protected pigeons, would have protected man It is a fact, however, that none of these rices contained 50 per cent of the external layers of the grain To show the relation between beri beri and the percentage of P_2O_5, the following Table has been prepared —

Table showing the relation between the Beri beri Factor and the Percentage of P_2O_5

P_2O_5 per cent	Beri beri Factor									Total
	0	0 01—0 50	0 51—1 00	1 01—1 50	1 51—2 00	2 01—2 50	2 51—3 00	3 01—3 50	3 51—4 00	
0 20—0 40	1		1	1	4		3	3	3	16
0·41—0 60	43	6	6	11	9	5	3			83
0 61—0·80	73			1						74
0 81—1 00	25									25
1 01—1 20										
1 21—1 40										
1 41—1 60	1									1
1 61—1 80	1									1
Total	144	6	7	13	13	5	6	3	3	200

Results of examination of fat in rices —The results of examination of fat in 200 samples chemically examined showed a wide variation, the figures ranging from 0 22 to 2 86 as maximum Pigeons fed on rices having at least 1 28 per cent

Java and *hand pounded* in Manila Out of these 10 samples *only 3 showed 1 05* per cent of ash or over while 7, or 70 per cent gave ash percentage ranging from 0 67 to 0 92 Notwithstanding this fact all the samples proved to be beri beri *preventing rices* The relation between the beri beri factor and the percentage of ash is given in the following Table —

Table showing the relation between the Beri beri Factor and the Percentage of Ash

Percentage of Ash	Beri beri Factor									Total
	0	0 01—0 30	0 51—1 00	1 01—1 50	1 51—2 00	01—2 50	2 51—3 00	3 01—3 50	3 51—4 00	
0—0 24										
0 25—0 49	1					1		1	[illegible]	5
0 50—0 74	5	1	5	3	7	3	5	[illegible]	1	3
0 75—0 99	41	4	1	10	6	1	1			64
1 00—1 24	57	1	1							59
1 25—1 49	37									37
1 50—1 99	3									3
Total	144	6	7	13	13	5	6	3	3	200

Phosphorus pentoxide —The P_2O_5 standard is better than the ash but is not nearly as good as the fat standard Out of 200 samples examined for P_2O_5 content 21 or 10 5 per cent was found to contain lower than the old 0 45 proposed standard for beri beri preventing rice and 179 or 89 5 per cent had the limit (0 45) or more In comparing these findings with the results of feeding experiments it was found out that the old standard 0 4 per cent is too low to be safe Pigeons fed on rices having a minimum of 0 6[illegible] per cent of P_2O_5 did not develop polyneuritis

A total of 99 samples of rice were found to have at least 0 62 per cent P_2O_5 and afforded complete protection At the same time there were 45 others that *coming below this minimum* afforded just the same protection On the other hand other rices having similar or relatively a higher percentage of P_2O_5 than the

old standard did not protect from polyneuritis, as is shown in the following Table —

Samples of rice producing polyneuritis in pigeons

Percentage of P_2O	0 4—0 49	0 50—0 59	0 60—61
Number of samples	43	27	2

It must not be forgotten that these experiments were made on pigeons, which are more susceptible to polyneuritis than man It is probable that certain rices with a high P_2O_5 percentage that have not protected pigeons would have protected man It is a fact, however that none of these rices contained 50 per cent of the external layers of the grain *To show the relation between beri beri and the* percentage of P_2O_5 the following Table has been prepared —

Table showing the relation between the Beri beri Factor and the Percentage of P_2O_5

P_2O_5 per cent	Beri beri Factor: 0	0 01—0 50	0 51—1 00	1 01—1 50	1 51—2 00	2 01—2 50	2 51—3 00	3 01—3 50	3 51—4 00	Total
0 20—0 40	1		1	1	4		3	3	3	16
0·41—0 60	43	6	6	11	9	5	3			83
0 61—0·80	73			1						74
0·81—1 00	25									25
1·01—1 20										
1 21—1 40										
1 41—1 60	1									1
1 61—1 80	1									1
Total	144	6	7	13	13	5	6	3	3	200

Results of examination of fat in rices—The results of examination of fat in 200 samples chemically examined showed a wide variation the figures ranging from 0 22 to 2 86 as maximum Pigeons fed on rices having at least 1 28 per cent

Java and hand pounded in Manila Out of these 10 samples, only 3 showed 1.05 per cent of ash or over, while 7, or 70 per cent gave ash percentage ranging from 0.67 to 0.92 Notwithstanding this fact, all the samples proved to be beri beri preventing rices The relation between the beri beri factor and the percentage of ash is given in the following Table —

Table showing the relation between the Beri beri Factor and the Percentage of Ash

Percentage of Ash	Beri beri Factor									Total
	0	0.01—0.50	0.51—1.00	1.01—1.50	1.51—2.00	2.01—2.50	2.51—3.00	3.01—3.50	3.51—4.00	
0—0.24										
0.25—0.49	1					1		1	2	5
0.50—0.74	[illegible]	1	5	3	7	3	5	2	1	3[illegible]
0.75—0.99	41	4	1	10	6	1	1			64
1.00—1.24	57	1	1							59
1.25—1.49	37									37
1.50—1.99	3									3
Total	144	6	7	13	13	5	6	3	3	200

Phosphorus pentoxide —The P_2O_5 standard is better than the ash but is not nearly as good as the fat standard Out of 200 samples examined *for P_2O_5 content* 21 or 10.5 per cent, was *found to contain lower than the old 0.45 proposed standard* for beri beri preventing rice and 179 or 89.5 per cent had the limit (0.45) or more In comparing these findings with the results of feeding experiments it was found out that the old standard 0.4 per cent is too low to be safe Pigeons fed on rices having a minimum of 0.62 per cent of P_2O_5 did not develop polyneuritis

A total of 99 samples of rice were found to have at least 0.62 per cent P_2O_5 *and afforded complete protection At the same time there were* 45 others that coming below this minimum afforded just the same protection On the other hand, other rices having similar or relatively a higher percentage of P_2O_5 than the

old standard did not protect from polyneuritis, as is shown in the following Table —

Samples of rice producing polyneuritis in pigeons

Percentage of P_2O_5	0 4—0 49	0 50—0 59	0 60—61
Number of samples	43	27	2

It must not be forgotten that these experiments were made on pigeons, which are more susceptible to polyneuritis than man It is probable that certain rices with a high P_2O_5 percentage that have not protected pigeons, would have protected man It is a fact however, that none of these rices contained 50 per cent of the external layers of the grain To show the relation between beri beri and the percentage of P_2O_5, the following Table has been prepared —

Table showing the relation between the Beri beri Factor and the Percentage of P_2O_5

P_2O_5 per cent	Beri beri Factor									Total
	0	0 01—0 50	0 51—1 00	1 01—1 50	1 51—2 00	2 01—2 50	2 51—3 00	3 01—3 50	3 51—4 00	
0 20—0 40	1		1	1	4		3	3	3	16
0·41—0 60	43	6	6	11	9	5	3			83
0 61—0 80	73			1						74
0·81—1 00	25									25
1·01—1 20										
1 21—1 40										
1 41—1 60	1									1
1 61—1 80	1									1
Total	144	6	7	13	13	5	6	3	3	200

Results of examination of fat in rices—The results of examination of fat in 200 samples chemically examined showed a wide variation the figures ranging from 0 22 to 2 86 as maximum Pigeons fed on rices having at least 1 28 per cent

of fat did not develop polyneuritis. Out of the total samples (200) examined 84 or 42 per cent fall below the 1·28 per cent fat and 116, or 58 per cent of the rices gave 1·28 or more percentage of fat, and all (116) afforded protection. The total pigeons that did not develop beri beri however, is 144. There are therefore, 28 more samples that although having less than 1·28 per cent of fat, did likewise afford protection. It is a fact, however, that percentage of fat (1·28), taken as a standard, would constitute a better index than the ash or P_2O_5 but practically less dependable than the per cent of pericarp remaining. The relation between the percentage of fat and the beri beri factor is shown in the following Table —

Table showing the relation between the Beri beri Factor and the Percentage of Fat

Percentage of Fat	Beri beri Factor									Total.
	0	0·01—0·50	0·51—1·00	1·01—1·50	1·51—2·00	2·01—2·50	2·51—3·00	3·01—3·50	3·51—4·00	
0—0·24					1					1
0·25—0·49					2	1	3	1	2	9
0·50—0·74			2	5	2	3		2	1	15
0·75—0·99	3	2	3	7	6	1	3			25
1·00—1·24	19	4	1	1	2					27
1·25—1·49	32		1							33
1·50—1·74	27									27
1·75—1·99	29									29
2·00—2·24	18									18
2·25—2·49	12									12
2·50—2·74	2									2
2·75—2·99	2									2
Total	144	6	7	13	13	5	6	3	3	200

In an effort to look for a more dependable and satisfactory standard that would exclude all rices, or at least the great majority of them that may produce beri-beri, it was tried to find out whether the summations of ash and P_2O_5 factors together, of P_2O_5 and fat together, and of fat ash and P_2O_5 and then of fat(2) plus P_2O_5, of fat plus P_2O_5 plus ash would make a more satisfactory standard The results of these trials showed that the total of fat, ash and P_2O_5 while it may be considered a better standard than all the rest excludes also beri beri protecting rices

A résumé of the values of the percentages of each one of these chemical compositions, as a beri beri preventing index is shown together in the following Table —

Table showing the values of the percentages of the different chemical components of rice as a beri beri preventing index

Factors considered	Minimum standard found in percentage or totals	Number of samples of protecting rice excluded	Remarks
Ash	1 05	59	
P_2O_5	0 62	45	Better than the ash but is not as good as the fat
Fat	1 28	28	Better than the previous ones
P_2O_5 + Ash	1 70	43	Slightly better than P_2O_5 alone
P_2O_5 + Fat	1 77	14	Better than fat alone
P_2O_5 + Ash + Fat	2 70	13	Better than the previous ones
2 Fat + P_2O_5	3 07	17	Less than P_2O_5 + Fat but better than P_2O_5 + Ash
2 Fat + Ash + P_2O_5	3 94	13	Not as good as the P_2O_5 + Ash + Fat

In coming to the selection of the best index for the standardization of rice, several factors should be considered, viz simplicity in the procedure, easiness in determination time employed in its determination, practicability of its application and other minor things to suit every particular locality and condition But, as a general index, the Committee may suggest the following, recommended by Colonel Vedder, thus —*'Any rice having 1·77 per cent of P_2O_5 plus fat but not less than 0 4 per cent P_2O_5 or any rice having not less then 0 62 per cent or any*

rice having not less than 0 5 per cent P_2O_5 and with at least 75 per cent remaining external layers ' One hundred and twenty nine rices, containing not less than 1 77 per cent of the totals of these constituents, afforded complete protection Out of this total, only one contained as little as 0 4 per cent of P_2O_5 It is to be observed that only nine out of all the samples that afforded protection to pigeons are excluded when the above requirements are possessed There is, therefore, no possibility of excluding, from the practicability view point, a large proportion of rice for having less than the required P_2O_5 percentage or other constituent

Classification of rice in its different stages in the process of milling—To formulate a more definite understanding in the designation and naming of the different degrees of milling of rice, the determination of the remaining pericarp by inspection and iodine staining is suggested as the most practical method, which, besides being convenient and suggestive of the facts regarding the incidence of beri beri, would make it possible to represent the degrees of *milling in relative figures The suggestion is to the effect that rices having 0 to* 20 per cent of the external layers remaining be called *highly milled rice*, those having 21 to 49 per cent *medium milled rice* and from 50 to 100 per cent, *under milled rice*

Effects of preparation of rice for food on the vitamin content—The different procedures used in different countries in the preparation of rice for food may and may not affect the suggested standard for rice as beri beri preventing We are not very familiar with the methods of preparing and cooking rice in other countries that common among the Chinese is to cook it with plenty of water as porridge rice, while others cook it with only enough water to cook and dry The Filipino way of preparing and cooking rice is in detail as follows —(1) Place rice in an earthen pot, (2) add enough water to cover the rice, (3) rub the rice against the inner sides of the pot for one to two minutes, (4) add more water to wash off the dirt and other suspended matters, (5) decant and throw all the water, (6) repeat operations (2), (3) (4) and (5) for two or three more times until the washing is almost clear, (7) add enough water to the level of about three or four centimeters above the surface of the rice, and (8) put on the fire to cook

Taking into consideration that the anti neuritic vitamin is freely soluble in water it may be presumed that rice treated in this way would readily lose part of its beri beri preventing power Experiments performed by the previous Committee on the P_2O_5 content of washed and unwashed rice, showed a reduction of this constituent after washing, the average difference in the ten samples examined being 0 25 per cent less in washed as compared with the unwashed *The practice* of rubbing the rice against the inner sides of the pot, as is the common way of cooking rice in the Philippines, instead of the mere washing alone used in the experiments, will undoubtedly remove a good portion of the external layers, and consequently reduce to a greater proportion the percentage of P_2O_5 content of the rice grains

The local method of preparing and cooking rice should always be taken into account as a factor of relative importance when we come to consider the local beri beri incidence

The index suggested for the standardization of rice provides a considerable margin of safety

Transportation and storage —Transportation of rice does not offer any problem in connection with the prevalence of beri beri except perhaps as regards the bags used in the transportation Paddy rice (palay) does not alter much whether packed in old or new, clean or dirty bags but milled rice needs to be packed for transportation in clean and insect free bags to protect the same from easy deterioration Rice during transportation especially in long voyages should be protected against moisture Fortunately in the Philippines inter island communication is not commonly long enough as to affect much the quality and keeping property of milled rice

On the other hand storage presents certain aspects which should be given consideration administratively speaking Paddy rice is usually stored not longer than nine months in the Philippines while rice after milling rarely remains longer than three months before it goes to the consumer As a matter of fact paddy rice under normal circumstances when in properly ventilated and wet proof store houses does not usually deteriorate after many months or even years There are different kinds of rice (palay) which deteriorate easily within a short time (garigan and others) but these varieties are raised in very insignificant quantities and only in certain localities of the islands On the contrary milled rice rapidly deteriorates if stored in damp and poorly ventilated places and if packed or stored in dirty old bags or insect-contaminated containers The degree of deterioration depends however on the kind of rice and on the degree of polishing and whitening to which it has been subjected in the milling process as well In the last report of the Beri beri Committee, the rapidity of deterioration has been the subject of detailed studies It was found out that under milled rice deteriorates earlier and more rapidly than over milled rice (within two months) while the latter can be stored from three to six months

The deterioration found consisted in the loss of the rice polishings the destruction of the germs and the kernel and the subsequent reduction of P_2O_5 content The most important factors found contributing to the deterioration of rice while stored were (*a*) the polishing itself due to its hygroscopic property (*b*) mites, (*c*) the rice weevil and rice beetle

While trying to find out the most suitable standard for beri beri preventing rice the following experiment was performed — Ten kilos of each sample of rice were purchased The rice was kept in tightly covered tin cans in a dry store-room each can being labelled with the serial number of the rice As experience promptly showed that weevils moth and other mites develop in rice so kept, a vial of chloroform with a loose stopper was buried in each sample

The escaping vapour promptly killed all insects and the rices kept in this manner remained in good condition during the 100 days that the experiment lasted

Whether the long storage and deterioration suffered there from affects or not the potentiality of beri beri preventing rice needs further studies and investigations Instances are there that prove that long stored under milled rice although musty and unfit for human consumption still prevented the development of polyneuritis in fowls when fed as an exclusive diet In a special series of experiments performed by Colonel Vedder, twenty deteriorated and heavily infected samples of rice were selected analysed and fed on pigeons The results were that none of them proved to be beri beri preventing rice It should be noted that seven out of the 20 samples contained originally 1 77 total of P_2O_5 plus fat which in accordance with the previous experiments should have prevented polyneuritis

Several methods have been suggested to prevent the deterioration of rice caused by insects *The use of carbon tetrachloride, of heat as it is being widely* used in the United States and of chloroform proved to be effective insecticides but none of these methods have as yet been tried on a large scale A sanitary regulation to keep rice mills in good clean and sanitary conditions providing also for the sterilization of rice bags and containers has been recommended by the Philippine Health Service at the suggestion of the last Committee but unfortunately very few municipalities have adopted them and the provisions are nowhere strictly enforced

IV Diagnosis of Beri beri

Several times in the course of the studies that have been made by the various committees the diagnosis of beri beri as stated in the death returns from the provinces has been questioned and doubt was aroused as to the correctness of the same and the reliability of the Philippine Health Service mortality figures on beri beri It was claimed that true beri beri cases are seldom seen in Manila (city) and the same condition might be occurring in the provinces By a resolution of the present Beri beri Committee it was decided that a clinician be appointed to conduct an investigation on the diagnosis of cases diagnosed as beri beri in the provinces Accordingly Dr Agerico B M Sison was appointed and given the following instructions —In order to have a more dependable basis on which to judge the beri beri situation in the Philippines especially in the provinces the diagnosis of beri beri in as many municipalities of several provinces as possible where beri beri prevails should be verified Verification of the diagnoses will be made on (*a*) cases of beri beri found in the dispensaries and puericulture centres both adults and infants (*b*) deaths from beri beri as stated in the death returns both adults and infants , (*c*) verify the errors in diagnoses separately in both cases (*d*) make a separate survey to see actually whether or not the disease is really increasing

The provinces of Nueva Ecija Cavite and Bataan, which appeared to have the highest rate of mortality and morbidity from beri beri, besides Manila, were chosen for this investigation Another physician Dr F Salud of the Public Wel fare Commissioner s Office helped Dr Sison in this investigation which was started on the 24th March in Manila and lasted until the 31st May in the province of Bataan

The towns of San Jose Muños Aliaga and Talavera were visited in Nueva Ecija the municipalities of Rozario Mendez Alfonso Bailen Kawit Noveleta Imus and Tanza were investigated in Cavite and in the province of Bataan the work was done in the towns of Balañga Pilar and Orani In the selection of these municipalities the high morbidity and mortality from the disease and the facilities of communication were taken into account

Results of the investigation

(a) *Manila* —Twenty seven cases were all the cases investigated in Manila during the short period of time available Out of this total 23 were among adults and four cases among infants Twenty four of this total (27) were confirmed giving an error of 11 1 per cent in diagnosis Out of seven deaths supposed to be due to infantile beri beri five were confirmed with an error of 28 58 per cent It must be said that all the seven cases of infantile beri beri were dead only 4 living cases having been found and 2 out of these having been confirmed The diagnoses in these cases were made on the clinical history of both the baby and the mother as found by actual and personal investigations

(b) *Nueva Ecija* —A total of 201 living cases and 18 dead of beri beri were investigated Out of 201 living cases 189 were among adults and 12 infants of which 140 cases in adults and 10 in infants were confirmed giving a total error of diagnosis in 25 37 per cent or 25 93 per cent and 16 67 per cent of error for adults and infants respectively Out of 18 deaths supposed to have been caused by beri beri in this province all among infants 15 were confirmed giving a correct diagnosis in 83 33 per cent and an error of 16 67 per cent

(c) *Cavite* —Eight municipalities have been visited in this province A total of 181 living cases and 25 deaths diagnosed as beri beri have been investigated Among the living cases only four were infants while among dead cases 23 were infants The errors of diagnosis found were 16 8 per cent in living cases and 56 per cent in dead cases All cases among infants were confirmed in 100 per cent while infants whose deaths were attributed to beri beri 52 12 per cent of the diagnosis was found incorrect

(d) *Bataan* —Very few cases and deaths from beri beri were investigated in this province due to the short period of time available There were in total 17 living cases and 22 deaths investigated The errors found were six per cent in living cases and 45 5 per cent in dead ones No living case was found among infants while out of the total 22 deaths diagnosed as beri beri occurring in

infants only 12 were found really due to beri beri giving an error of 45 5 per cent in diagnosis A résumé of the findings and errors is given in the following Table —

Errors found in the diagnosis of beri beri

	Manila			Nueva Ecija			Cavite			Bataan		
	No investigated	No confirmed	Percentage of error	No investigated	No confirmed	Percentage of error	No investigated	No confirmed	Percentage of error	No investigated	No confirmed	Percentage of error
Living Adults	23	[illegible]	4 49	189	140	25 93	180	149	17 [illegible]	17	16	6 00
Living Infants	4	2	50 00	12	10	16 67	4	4	0	0	0	0
Living Total	27	24	11 2	201	150	25 37	184	153	16 8	17	16	6 00
Dead Adults	0	0	0	0	0	0	[illegible]	0	100	0	0	0
Dead Infants	[illegible]	5	28 6	18	15	16 67	23	11	52 17	22	12	45 46
Dead Total	7	5	28 6	18	15	16 67	25	11	56 00	22	12	45 46

The findings obtained in Manila and three different provinces discard any doubt that errors of diagnosis are frequent especially in the death certificates Taken as a whole in the three provinces the diagnoses were found incorrect in 40 63 per cent of the deaths and 20 64 per cent of living and actual cases But the wide variation of errors found does not give the gauge of these errors nor allow the Committee to formulate an acceptable standard for the same that can be applied to our death returns and obtain a corrected death from beri beri It is to be considered further that the number of cases investigated in each province was scarce the provinces visited very few that it is not believed they constitute a representative number enough to draw a conclusion therefrom One fact however, had become known to the Committee and this was that in actual living cases personally seen by health officers in the dispensaries a relatively small percentage of error 20 64 per cent was detected as compared with the errors found in the diagnoses stated in the death certificates We have to repeat and remind here the fact that the majority of the death certificates in the provinces are prepared by laymen and the diagnosis stated therein has had to be based on the history of the disease and a few data given by the informant who in the majority of the cases being a mere family friend or neighbour might have not even seen the case

Another thing that the Committee cannot but over emphasize is the fact that, no matter how great the error found was in the diagnosis of beri beri in the death returns, the importance of the beri beri problem in the Philippines, as a health problem cannot be minimized nor under estimated The error found in the diagnosis of fatal cases in the three provinces was 40 63 per cent, if applied to our mortality figures in the provinces for the last few years they would show that beri beri in the provinces has, as was stated, been increasing The following Table of mortality from beri beri in the provinces, from 1910 to 1917 uncorrected, and from 1918 to 1926 inclusive corrected, on the basis of 40 63 per cent error, is given for information —

Years	Uncorrected	Years	Corrected
1910	4 128	1918	7 045
1911	4 367	1919	7 114
1912	4,372	1920	7 410
1913	3,194	1921	9 090
1914	4 102	1922	9 642
1915	4 336	1923	10 341
1916	5 874	1924	10 883
1917	7 463	1925	10 653
		1926	11 083

V COMMON DIET OF FILIPINO LABOURING CLASS

The Filipino labouring class is the group of the population mostly affected by beri beri The investigation of the last Committee showed that 89 18 per cent of the cases of beri beri occurred among the poor class of the population Our labouring class has a very meagre earning and therefore they cannot be expected to get a varied and more balanced diet If we admit that beri beri is a vitamin-deficiency disease as it is the general consensus of opinion, it has to be admitted, or at least it should be expected that, beri beri must be a prevailing disease among our people of the poor class taking into consideration their poor salary and that

rice is the staple diet The estimate of the daily cost of living in various provincial capitals according to data obtained from the Bureau of Labour, gives the following amount for food for the different years —

Daily cost of food

Years	1910	1918	1920	1925
A single labourer	P 0·43	P 0·65	P 0·84	P 0·71
A family of two adults and three minors	P 0·66	P 1·21	P 1·42	P 1·25

The daily cost of food for a family of two adults and three minors in various localities in the Philippines was also given by the Bureau of Labour as follows —

San José Antique	P 1·01	Iloilo, Iloilo	P 1·48
Legazpi, Albay	P 1·58	Laoag, Il Norte	P 0·97
Cebu Cebu	P 1·28	S Fernando, Union	P 1·02
Davao Davao	*P 1·55*	*Average*	P 1·28

It would be worth mentioning also, that the wage-earner population in the Philippines is estimated (Bureau of Labour) at 2,857,401 which is about 25 per cent of the total population of the Philippines The above facts would only show that *the population exposed to the risk of contracting beri beri is too big to expect a* lower incidence of beri beri in the islands

In the investigation of 600 families with a history of beri beri among their members it was found out by the last Committee, that the number of staples of diet, besides rice, which were most commonly consumed, may be reduced to seven varieties *It was also found out that the common diet of families with beri beri* cases among their members is not at all deprived of the anti neuritic vitamin factor ' On the contrary, the varieties of food more commonly consumed appear to be relatively rich in this substance In spite of this fact, the rate of beri beri incidence is high ' *Was there an insufficient amount of each variety of food* ingested and consequently *an insufficient vitamin for the requirements of the* metabolism ? Or are there other factors that should be accounted for in the causation of beri beri among the members of the investigated beri beric families ? These were the two questions made by the last Beri beri Committee for which it was recommended that further studies and investigations be performed

Taking advantage of the *investigations to be performed in various provinces* for the verification of diagnosis Professor F O Santos offered himself to work and study for the Committee in this respect Professor Santos visited three provinces, Nueva Ecija Cavite and Bataan, and with the co operation of one

assistant, made a quantitative and qualitative studies of the common diet of beri beric families as compared with that of non beri beric families Unfortunately, not having finished his experiments on the different varieties of food he had not been able to submit his report in time to be included here However, Professor Santos has apparently come to the conclusion that the diet of beri beric families although composed of different varieties rich in anti neuritic vitamin is insufficient in amount to supply the actual needs of vitamin It must be said that in the previous investigation performed the individual food habits of the members of families investigated have not been recorded it may be that those who came down with the disease are sparingly supplied with the vitamin containing food even when such articles of diet were available We personally know of several rich families in which one or two members had beri beri due to their individual likings of vitamin deficient foods As soon as the report of Professor Santos is submitted, the same will be published as an appendix to this report

VI Educational Campaign—Conferences Lectures Pamphlets

Following the recommendation of the previous Committee and the resolution of the last Congress of the Far Eastern Association of Tropical Medicine the Committee on beri beri with the funds available for the purpose started an educational campaign for the spreading of knowledge of the most important facts about the causes the prevention and treatment of beri beri The Philippine Health Service through its medical officers are co operating with this work The work performed in this respect during the last year was as follows —

(*a*) Publication of some hints on beri beri prevention and ætiology in the daily papers

(*b*) Cinematographic projections on the prevention and causes of beri beri (translated into different local dialects)

(*c*) Conferences on the same subjects given to the teachers in Baguio

(*d*) Conferences on the same subject in the towns and barrios by *Presidents* of Sanitary Divisions and District Health Officers as part of their duties

(*e*) Publication of a pamphlet on the ætiology symptoms and prevention of beri beri This pamphlet is being translated into different dialects

VII Tiki tiki Production

The tiki tiki production in the islands has not increased during the last few years Tiki tiki extract is the only product known by the people to cure beri beri and it is the most commonly used The Beri beri Committee has again recommended the purchase of enough material and machineries to increase production, for free distribution

VIII. Summary

1. Beri-beri is a prevailing disease in the Philippines. It is decreasing in Manila, but slightly increasing in the provinces.

2. The importation of rice has relatively increased during the last three years, although our local production has also increased.

3. Correlation exists between the local production of rice and the incidence of beri-beri.

4. Beri-beri prevails during the months of October, November, December and January.

5. Beri-beri is widely distributed in the islands, although there is a wide variation in the rates of mortality.

6. The proportion of the external layers remaining on a given rice may be determined with reasonable accuracy by inspection after staining with Gram's iodine solution.

7. Rices examined by inspection method, after staining having 50 per cent or more of the external layer, do not produce polyneuritis when fed to pigeons.

8. Selection of rice by using the minimum, 50 per cent external layers remaining as standard through staining and inspection method, may prevent beri-beri.

9. This method may be used for the classification and naming of the different stages of rice during the process of milling.

10. Amido nitrogen is useless as a chemical index, 1.05 per cent ash is a poor index, 0.62 per cent P_2O_5 content is better, and 1.28 per cent fat is a much better index.

11. Rice having 1.77 per cent P_2O_5 plus fat but not less than 0.4 per cent P_2O_5 content, or rices having not less than 0.62 per cent P_2O_5 or rice having not less than 0.50 per cent P_2O_5 and with at least 75 per cent of the external layers, proves to be a beri-beri preventing rice in pigeons. These rices excluded only 9 rices out of 200 that afforded protection to pigeons.

12. Rice becomes deteriorated while stored and the causes of deterioration are mainly dampness and insects.

13. Under-milled rice deteriorates earlier and more rapidly than the over-milled rice.

14. The different trial methods of preparing rice for food affect the P_2O_5 and presumably the vitamin content.

15. Errors of diagnosis of beri-beri in the city and the provinces not only in living cases but also in fatal cases, were found. There is wide variation in the errors found in different localities.

16. No matter how great the error found was, there is no doubt that the problem of beri-beri is of capital importance in the islands.

17. Our labouring and poor classes are the most affected by beri-beri.

18 The average daily cost of food for a family of two adults and three minors is P 1 35 This amount is considered too small to permit an abundant food

19 The diet of beri beric families as found by the investigation, although varied, seems to be inadequate in amount

IX RECOMMENDATIONS

1 Eighty per cent remaining external layers of the grain of rices determined by staining and inspection method may be recommended as standard for the selection of rices for institutions and armies This is not recommended as a legal standard

2 Rices with 0 to 20 per cent of the external layers should be called *highly milled rice* Those having 21 to 49 per cent *medium milled rice* and those having 50 to 100 per cent *under milled rice* In the determination of the percentages of remaining external layers the Gram iodine staining and inspection method should be used

3 The production of highly milled rice should be discouraged

4 Any rice having 1 77 per cent of P_2O_5 plus fat, but not less than 0 4 per cent P_2O_5 or any rice having not less than 0 62 per cent P_2O_5 or any rice having not less than 0 50 per cent P_2O_5 and with at least 75 per cent of the external layers of the grain remaining is suggested as the tentative chemical index

5 The production of vitamin containing home vegetables should be encouraged

6 A wide campaign of education for the spread of knowledge about beri beri prevention should be continued

THE CAUSATION OF LATHYRISM IN MAN

BY

LIEUT COL HUGH W ACTON, I M S,
Professor of Bacteriology and Pathology

AND

LIEUT COL R N CHOPRA, M A, M D (Cantab), I M S,
Professor of Pharmacology,
Calcutta School of Tropical Medicine and Hygiene

IN 1922 we investigated lathyrism both in the field and by laboratory methods at that time we considered that this disease was due to eating *khesari dal* or *Lathyrus sativa* We thought that the poison was present in the greatest amount in grain that was capable of germinating and as the cases of lathyrism usually developed during the end of July (Asar) that perhaps during the monsoons the grain may have undergone germination Our error arose from the fact that we were medical men and not botanists because we submitted the different types of contaminating seeds to an expert for identification Amongst the various seeds sent was a small black grain called in Rewa *akri*, this was identified for us as *Withania coagulans*, a harmless seed that had an enzyme like rennet and is sometimes used by the Indians to coagulate milk In January 1923 at the Indian Science Congress at Lucknow Dr Howard pointed out to us that this *akti* or *akri* was not *Withania coagulans* but a weed *Vicia sativa* In 1925 Howard Simonson and Anderson published their paper concluding that *khesari dal* (*Lathyrus sativa*) did not contain any poisonous bases at all and did not produce symptoms of paralysis in ducks that had been fed exclusively on this vetch provided it had been freed from *akti* (*Vicia sativa*) When *Vicia sativa* was given to ducks and monkeys in the proportion of 10 to 50 per cent in their food it produced symptoms of the nervous system followed by death At first their work did not appear to us to be very convincing as the symptoms we had observed in our experimental animals were very slight indeed merely paralysis of the legs whilst their birds had the neck muscles wings etc paralysed and this paralysis was followed by death Moreover they laid great stress on the fact that divicine was closely related to barbituric acid as being the source of the poison but one can hardly call a substance that can only produce symptoms in animals (guinea pigs) at a dose of 0 6 milligrams per

gramme of body weight to be the toxic substance According to this estimate 42 grammes of divicine would be required to produce symptoms in a man of 70 kilos In 1927 McCombie Young acknowledged that when this legume *Lathyrus sativa* predominates in the diet lathyrism results and that such a community is also in a state of marked instability due to the lack of protective substances notably those containing fat soluble vitamin A He tentatively suggests that lathyrism may be to some extent a deficiency disease These three theories all agree on two essential points (1) that a diet of *Khesari dal* (*Lathyrus sativa*) of over 50 per cent mixture is a dangerous diet (2) that this diet must be taken for about three months before symptoms appear These two observations were made in 1904 by Buchanan who had a considerable practical experience of this disease which the other observers including ourselves have not had We will, therefore first consider the vitamin A theory that McCombie Young has recently announced, as the results he has published have caused the authorities to include our paper in this section on deficiency diseases No one can deny that a diet consisting wholly of *Khesari dal* day in and day out is not only a monotonous as well as a deficient diet and the same can be said of the *chunna* diet (*Cicer arietinum*) when taken with only salt or unrefined sugar (*ghur*) Both these diets would cause night blindness but not lathyrism

When there are two theories i e a toxic and an avitaminosis theory co existing side by side there is generally a certain amount of truth in both theories On the one hand there is a poison which is the actual causation of the disease and on the other hand the natural defence mechanism of the body against such poisons and the liberal supply of vitamines raises this natural defence mechanism When the poison is present in such large quantities that the defence mechanism is not sufficiently strong to overcome it then every member of the household or the community is affected by the disease When the poison is present in the food in only moderate or in small quantities and the defence mechanism is affected by the lack of vitamins the disease will make its appearance amongst those few members of the community whose defence mechanism is not up to normal According to the followers of the vitamin theory as a causation of a disease like lathyrism the right procedure would be to give these people a sufficient quantity of fat soluble vitamin A and so prevent the disease Unfortunately the sufferers of this disease are miserable bondmen who have to live on *Khesari dal* during famine times in spite of the views of the laboratory Both sides are arguing about facts that are partially correlated with the causation of the disease and few research workers realize what these incomplete correlations really mean in explaining the whole truth of a disease The object of a research is always to find out the truth irrespective of whether it means siding with one side or the other and when mistakes are made to renounce one's error

We will now proceed to acknowledge our own errors made in 1922 during the course of our investigation After our return from Lucknow in 1923 and having heard the conjoint paper by Howard Simonson and Anderson we realized that

akti or *akri* was not *Withania coagulans* but *Vicia sativa* We next investigated the different samples of the grain that we had been working at, and found —

(1) The amount of *akti* (*Vicia sativa*) present in the different samples of grain that we had tested was as follows'—in some samples there was no *akti*, and in others we obtained as much as 2 grammes per kilo of *khesari dâl*

(2) More *akti* was present in the Bhagalpur grain This we found to be germinating grain, and has been incriminated by the people as causing lathyrism In the non germinating samples, i e, when cut unripe, obtained and grown in Rewa little or no *akti* was found as an adulterant

(3) The grains of the *Vicia sativa* are not so easily recognized as we had been lead to think in the accounts related to us by the local people They stated that the *akti* was always separated from the *khesari dâl*, and therefore we did not regard it of any importance as an ætiological factor in this disease

Dr Howard had already informed us of the danger when working with impure samples In the meantime as we could not get pure samples we applied to *Dr S N Bal of the Indian Museum, who has supplied us with typical samples of the* grain of this seed and we are extremely grateful to him for all the help he has given us in this investigation These typical samples enabled us to sort out roughly the seed of the *Vicia sativa* by recognition and not, as Dr Howard had done, by growing the seeds and separating the *vicia* plants from the *khesari* by weeding which is the ideal method In spite of this rough method of purification, we re examined the whole of our work again and found —

(1) That non germinating (*Lathyrus sativa*) seed freed of *akti*, and it is generally free of this weed, contained no alkaloid or toxic substances when injected into guinea pigs The grain we obtained from Rewa and which we spoke of as non germinating grain is due to the fact that the *khesari* is cut before the seeds are completely ripe and hence the failure in germination

(2) The seeds of *Lathyrus sativa* that have germinated for 48 hours and previously freed from *akti* contained no alkaloids or toxic substances when injected into animals In our previous work when the *khesari* was not freed from *akti* we were able to find these poisonous bases

(3) Ducks fed on germinating and non germinating *Lathyrus sativa*, that were freed from *akti* showed no symptoms of lathyrism after feeding them on this diet for 60 days or more, although at the end of the experiment we would not consider these ducks to have been especially fed for the table

When the samples of grain were taken containing about 1 5 grammes of *akti* to the kilo of *Lathyrus sativa*, we found that —

(1) The germinating grains of *Lathyrus sativa* contained the most *akti* as an adulterant, and when these toxins were separated chemically we were able to produce in guinea pigs paralysis of the hind limbs with doses of 50 milligrams of the impure base The guinea pigs recovered from these paralyses within two days

(2) Ducks fed on *Lathyrus sativa* contaminated with *Vicia sativa*, i e, only 2 grammes to the kilo, after 45 days showed symptoms of paralysis Out of 10 ducks

fed in this manner two showed symptoms of paralysis of the legs and two were paralysed to the extent depicted by Howard Simonson and Anderson's paper when they used fairly large quantities i e 10 to 50 per cent of *akti* as an adulterant in their feeding experiments

There can be little doubt from the results of these experiments that the error in our original tests was due to the fact that we regarded this weed as *Withania coagulans* and considered it a harmless adulterant

The next point we have to consider is the nature of these chemical constituents of *Vicia sativa* We have prepared divicine synthetically and find that it has very little toxicity such as has been claimed by Simonson in the above paper When we injected 0 6 milligrams by gramme weight of the animal the amount of drug caused necrosis either by its concentration or acidity Now divicine is closely related to barbituric acid which forms a toxic principle in many drugs such as veronal luminol and propinol and the very toxic dial which is dialyl barbituric acid Ten milligrams of the latter compound is lethal for a kilo weight of a rabbit Divicine can be split into barbituric acid by acid hydrolysis when the two NH_2 groups are replaced by an H and O group It is probably due to this hydrolysis of barbituric acid that some toxic substance is formed which acts on the central nervous system Therefore in the main we agree with Howard Simonson and Anderson that the cause of these paralytic symptoms produced in experimental animals is due to —

(1) The contaminating weed *Vicia sativa* and not to any poisons in the *Khesari dal* or *Lathyrus sativa*

(2) We found in epidemic dropsy that the whole of the intestinal flora is altered when this disease is active and it is possible that a similar state of affairs may occur in lathyrism so that some toxic substance is produced from the divicine as the result of hydrolysis taking place in the gut

(3) That when a deficient diet such as a whole diet of *Khesari dâl* is given to man the general resistance of the body is lowered so that these poisons can act more powerfully on such individuals

We therefore believe that these facts explain all the conflicting results that have occurred in the laboratory researches and in the field work In Rewa during ordinary years without famine when most of the *Khesari dal* that is eaten is free from *akti* very little lathyrism occurs In famine years when the Bhagalpur *dal* is imported containing a fair amount of *akti* lathyrism is much more common In such years the diet on which these bondmen live is wholly made up of *Khesari dal*, as it is the cheapest food that is available at a time when prices are high Owing to the failure of the rains practically no other food i e green vegetables milk or even butter milk is available for these people

The prevention of this disease turns on —(1) the improvement of the economic condition of these people and the abolition of the system of bondage if the latter still exists in this State.

(2) That *Khesari dal* as Dr Howard suggests should be planted in drills and the contaminating *Vicia sativa* removed by the people by weeding This should

not only be done in places like Rewa where lathyrism is rife, but also in those areas from where the imported grain is sent to the famine stricken areas

The separation of *Vicia sativa* as Dr Howard points out, can easily be under taken by the people themselves as soon as they know the danger of this weed The improvement in economic conditions depends on utilizing the mineral wealth of the country for at present very few concessions are given to outsiders to work the mineral wealth such as limestone etc

REFERENCES

ACTON (1922)	An investigation into the causation of lathyrism in man *Ind Med Gaz*, Vol LVII, No 7 July
ACTON and CHOPRA (1922)	The production and pharmacological action of khesari amine *Ibid*, No 11 November
Idem (1925)	The problem of epidemic dropsy and beri beri *Ibid* Vol LX, No 1, January
Idem (1927)	Further investigations into the ætiology of epidemic dropsy *Ibid*, Vol LXII No 7 July
ANDERSON, HOWARD and SIMONSSON (1925)	Studies on lathyrism *Ind Jour Med Res* Vol XII p 613
BUCHANAN (1904)	Report on lathyrism in the Central Provinces
HOWARD, SIMONSSON and ANDERSON (1923)	*Ind Jour Med Res*, Vol X, p 857
LEVENE, and SENIOR (1916)	*Jour Bio Chem*, Vol XXV, p 607
TRAUBE (1893)	*Ber d deut Chem Ges*, Bd. 26, p 2556
McCOMBIE YOUNG (1927)	*Ind Jour Med Res*, October

DISCUSSION

Lt Col R McCarrison, I M S (B India) Col McCombie Young has asked me to precede him in the remarks which he has to make and to give you the results of my experiments designed to produce lathyrism by means of four seeds

(1) Pure *Lathyrus sativus*, large seeded variety

(2) Pure *Lathyrus sativus* small seeded variety

(3) Pure akta or (*Vicia sativa*)

All grown by Mr Howard at the Botanical Institute at Indore None of these grains cause any disease resembling lathyrism in *rats*

Similarly, the Lathyrus actually eaten by sufferers from the disease did not cause lathyrism in rats

Lieut -Col T C McCombie Young I M S (B India) My chief reason for intervening in the discussion is to represent that Col Acton has apparently misunderstood me in regard to *akri* in the diet of lathyrism cases, for the main thesis of my paper was to the effect that *akri* contamination was so rare and so slight in amount in Rewa that it could have but little, if any effect in producing the symptoms of lathyrism I was driven to this conclusion despite an initial pre possession in favour of the *akri* theory because the further one went the clearer it became that *akri* contamination was unimportant As the result of a considerable amount of research I obtained specimens of the grain which recent cases of lathyrism had been eating before they fell ill Six of these specimens

were sent to Dr Howard at the Institute of Plant Industry for examination Out of them four contained no *akri* at all and two, which were the residue remaining at the bottom of a storage jar (Sautuh) contained a few *akri* seeds which had fallen to the bottom of the jar and were in no way indicative of the proportion of contamination in the grain eaten Subsequent specimens, which were sent to Lieut Col McCarrison obviously contained no *akri* and were not specially examined for it Yet these were samples of the grain which had formed the food of recent lathyrism cases

Lathyrism is a famine year phenomenon when, on account of the failure of the rains and resulting dryness of the soil, the wheat crop fails to germinate and only the lathyrism crop comes up The villagers point out that *akri* grows only in wet fields and when the fields are too dry for wheat no *akri* comes up How then can it be a cause of lathyrism in famine years when such cases are common? Other evidence which I collected all yielded the same inference The point I wish to make to-day is that cases of lathyrism occur in persons eating Lathyrus in which there is no *akri* at all or, if there is any such contamination, it is so rare and so small in amount as to make it unlikely, from the facts as I saw them, that an admixture of the seeds of *Vicia sativa* plays any part in the production of lathyrism.

In reply to Col Acton's subsequent remarks

With regard to the possibility of imported 'Bhagalpore matra' in a famine year playing any part in the production of the large number of cases of lathyrism of such a year, I may say that I inquired into the dietary histories of a large number of famine year cases, and quite a number of them had acquired lathyrism when eating the Lathyrus which was the product of their own fields, i e, the large seeded variety, which is not ordinarily contaminated with *Vicia sativa* nor in such a year is it likely to be so contaminated

From these histories it appeared that both the home grown and the imported grain were equally likely to be associated with the onset of the disease

SALT LICKS

BY

MAJOR CLIVE NEWCOMB, I M S,

Chemical Examiner to the Government of Madras

A VERY widespread habit amongst the larger animals is to resort at interva to certain places in the jungles and there lick or eat the earth This has le to the common assumption that they go there to seek some necessary constituent of their diets which they cannot obtain elsewhere, and more particularly—sodiu chloride It was thought that this assumption might be worth testing and furthe that some interesting information might possibly be gained as to what miner constituents are necessary in the diet of the larger animals, by the analysis of th earth from these 'salt licks'

The collection of material is a difficulty, but by the kindness of various fores officers and others, samples of soil from seven authenticated salt licks were got Th general analyses of these are shown in the following Table —

Number	I	II	III	IV	V	VI	VII
Loss on ignition	6.7	8.9	10.1	5.8	9.7	12.1	4.1
Sand and other insoluble substances	76.9	65.2	66.4	70.3	73.0	66.7	72.2
Silica as SiO_2	7.4	5.0	7.0	3.0	6.0	7.7	8.1
Ferric oxide	3.6	4.9	9.4	8.5	5.7	4.8	5.4
Alumina	6.4	10.6	0.6	2.6	7.3	10.1	7.6
Lime as CaO	None	5.3	5.0	10.8	Trace	Trace	2.5
Chlorides as NaCl	0.052	0.27	0.094	0.016	0.010	0.177	0.011
TOTAL	101.1	100.2	98.6	101.0	101.7	101.5	99.9

Licks I and II are in the plains about 30 miles from Madras

The others are in various places about the Nilgiri Hills

It is clear from these figures that the amount of sodium chloride that an animal could get, even if it were to eat a considerable meal of any of these soils is negligible,

so that whatever else they may go for it is not for sodium chloride It has been suggested that it is for sodium itself—and not for any particular salt of it—that the animals go to these salt licks It is said that it is only herbivorous animals which eat the earth at salt licks because eating plants their diet contains an excess of potassium and they have to seek sodium specially to counterbalance this The chemical determination of sodium is difficult and Bertrand and his co workers have recently shown that the older determinations of sodium in plants are frequently inaccurate and that all the plants they have examined many of which were formerly considered to contain no sodium do in fact contain this element It is therefore questionable if the alleged excess of potassium over sodium in herbivorous animals diets is a fact In the above analyses the sodium was not estimated but as the total of the constituents found adds up to 100 per cent within the experimental error, there cannot be much sodium present and it seems very doubtful if there could be enough to make it worth their while to go and get it A new precipitant for sodium is under trial and if and when the determination works properly the sodium in these soils will be determined The presence of only a trace or no calcium in three of them excludes this element as the substance sought They are all fairly rich in iron so that this is a possibility though it seems unlikely

The soils were also examined for their content of iodine a necessary element in animals diets and one that wild animals might well have to seek out specially The results were —

Salt lick	Iodine content Parts per ten millions
I	49
II	20
III	2[illegible]
IV	45
V	3
VI	4[illegible]
VII	7
Blank	0

Most of them therefore contain considerable iodine but licks III to VII come from the Nilgiri Hills and the soil of this part of India is particularly rich in iodine In three analyses of the ordinary soil of these hills 238 310 and 250 parts of iodine per ten millions of soil were found It seems therefore that if it is iodine that the wild animals want they would do better to eat the ordinary soil of the jungles in which they happen to find themselves instead of making a special journey to a salt lick to get it

The reason for the animals going to these salt licks remains a mystery, but the problem seems to the writer an interesting one, and this inconclusive note is offered for publication in the hope of interesting others in the question and so obtaining further samples of salt licks for analysis

The writer is indebted to General Symons for suggesting the investigation, to Mr T R Subrahmanya Ayyer, his second assistant, for help in the general analyses and to Mr G Sankaran his research assistant, for help in the iodine determinations

REFERENCE

Bertrand, C and Lfrietzfanl, J (1927) C P 181 615

DISCUSSION

Mr J T Edwards (United Provinces) I should like to ask the speaker if in the analysis of the soils of the salt licks he investigated the proportion of phosphorus (P_2O_5) in the licks (Answer No) This investigation would seem to be very necessary for the reason that, as was stated by Col McCarrison yesterday, a marked deficiency of phosphorus in the soil, and hence in the fodder, underlies the causation of the South African cattle disease lamziekte The craving or pica of the cattle can be thoroughly satisfied and the animals cured of the addiction and the lamziekte then indirectly effectively prevented by the administration to the cattle daily of a small quantity of lick containing powdered bone meal Again it does not seem to me that the speaker has paid sufficient attention to the high content of iron discovered by him in the soil of the licks It is now well known that iron deficiency plays a very important part in bringing about metabolic derangement in the domesticated animals in a number of territories For example, in the Northern Island of New Zealand outbreaks of grave trouble occur among flocks of sheep kept on land that is exceptionally poor in iron and the trouble can be prevented by the administration of some substance, akin to Parrish s food containing iron Lately, Orr and his colleagues at Aberdeen have carried out experimental work to show the great importance of iron in the dietary of pigs

The subject brought up is one of immense importance in the livestock economy of India and deserves careful study

Major C Newcomb I M S (Madras) replied Mr Edwards has raised a very good point in suggesting that it may be phosphorus that the animals seek in these salt licks The suggestion will be investigated

THE EXPERIMENTAL PRODUCTION OF LYMPH ADENOID GOITRE

BY

LIEUT COL R McCARRISON, C I E, M D D SC, LL D F R C P I M S,
Director Deficiency Diseases Inquiry Indian Research Fund Association

I HAVE ventured to introduce this subject to the notice of the Conference since the type of goitre with which it deals falls within the realm of deficiency diseases '

In 1925 Williamson and Pearse described a type of goitre in man which they designated Lymph adenoid Goitre ' its cause was unknown This communication deals with its experimental production in rats

Lymph adenoid goitre is the hypertrophic reaction of a physiologically insufficient organ with which there occurs a preponderance of lymphocytic aggregates fibrosis and a peculiar atrophy of the parenchyma The condition can be focal and non goitrous (Williamson and Pearse 1925)

Macroscopically the experimentally produced goitres are of glistening fleshy appearance bright or darkish red in colour and of a small size being from 50 to 300 per cent larger than the normal organ The isthmus is usually prominent thickened and broadened Commonly one lobe is more enlarged than the other occasionally the enlargement is unilateral Microscopically it is characterized in its progressive stage by intense secretory hypertrophy almost approaching the complete heterotrophy of the adenoid goitre of primary Graves's disease small non papilliferous secretory follicles and pervasion of the gland units by lymphocytes In its retrogressive stage when failure of the gland's compensatory mechanism takes place, it is characterized by exhaustion of more or less of the epithelium, collections of round cells and fibrosis with the production of a state identical with that to which the term *lymph adenoid goitre* has been applied These changes may be present without any marked increase in size of the gland or they may be of focal distribution

The basal factor in the causation of this type of goitre is a diet from which green vegetables and fruit are excluded containing more than 60 per cent of white flour or vitamin poor carbohydrate 20 per cent or less of protein 8 to 10 per cent of vitamin poor fats and inorganic salts (including iodine) in adequate amounts It occurs in approximately 25 per cent of rats so fed and is more common in females than in males It is unrelated in its origin to iodine deficiency , some animals

acquiring it had consumed from 0 4 to 2 6 mg of potassium iodide with their food daily while others had received no additional iodine

The diet with which this type of goitre is associated is deficient in vitamins of the A B and C classes and as such it would be likely to induce a physiologically subnormal state of the thyroid gland and to lead to disturbances of metabolism with the production within the body of toxic metabolites It is liable also to induce a physiologically subnormal state of the gastro intestinal tract with intestinal stasis and to favour intestinal infection and 'toxic' absorption from the bowel It seems probable therefore, that the pathological changes which result in the thyroid of certain individuals from the faulty food are the evidence of aberrant attempts at compensatory hyperplasia in an organ rendered physiologically inefficient by imperfect nutrition or of the action upon it of metabolic or intestinal toxins' or more probably still, of both together

In view of the almost universal use of white flour as the main staple of the dietary in Western countries of the widespread use of vitamin poor carbohydrates and of substitutes for butter which are relatively poor in vitamin A and of the scanty use of fresh fruit and green leafy vegetables it seems probable that this type of goitre will be found to occur sporadically, or even in endemic proportions amongst Western peoples, that it will be encountered in its progressive stage in childhood and in young women whose food contains much vitamin poor carbohydrate little suitable protein and vitamin A containing substances and less green vegetables and fruit that its retrogressive stage will be found in older subjects whose food has had similar faults since childhood, that the subjects of its progressive stage will be prone to develop Graves's disease following such influences as fright mental worry, pregnancy lactation and attacks of acute infectious disease and that the subjects of its retrogressive stage will exhibit greater or lesser degrees of hypothyroidism If these predictions should prove to be correct then it will be found that the additional provision of iodine in the food will neither prevent nor cure this type of goitre but that a well balanced vitamin rich diet will

RELATIONSHIP OF IODINE IN SOIL AND DRINKING WATER TO THE CHRONIC HYPERTROPHIC TYPE OF ENDEMIC GOITRE

BY

LIEUT COL R McCARRISON, C.I.E., M.D., D.Sc., LL.D., F.R.C.P., I.M.S.,
Director, Deficiency Diseases Inquiry, Indian Research Fund Association

THE parenchymatous or chronic hypertrophic type of endemic goitre is the classical 'goitre' of mountainous regions such as the Alps and Himalayas. It occurs in association with cretinism, deaf-mutism and various forms of physical and mental deterioration. Because of its confusion with other types of simple goitre—of which there are several—controversy regarding its ætiology has raged since the time of Hippocrates. Some 60 or 70 years ago it was thought to be due to deficiency of iodine in the soil, drinking water and food. This view was abandoned but has been vigorously revived within the last 10 to 15 years. My own observations during the past 25 years have demonstrated its relationship to bacteriologically impure water supplies.

In this communication I propose briefly to summarize the result of an investigation of the relationship of this type of endemic goitre to iodine carried out by Newcomb, Norris, Viswanath and myself during 1925-1926. The results are these :—

In sea coast, sub-montane and montane localities (ex-Himalayan India) where endemic goitre is unknown, the iodine content of the soil may be very low (less than 5 parts per 10 millions), moderate in amount (25 to 45 parts per 10 millions) or high (100 to 400 parts per 10 millions). The freedom of these localities from goitre is therefore related to conditions of life other than the iodine content of their soils.

The iodine naturally present in the soil cannot easily be washed out of it by rainfall or by floods.

The richness of a soil in iodine (100 to 400 parts per 10 millions) does not preclude the presence of thyroid swellings, for the most part the so-called 'incipient goitres'—in 27.7 per cent of girls and in 11.8 per cent of boys living at altitudes of 6,000 or so above sea-level in places where endemic goitre and its sequelæ (cretinism, etc.) are conspicuous by their absence in the general population. Nor does it preclude the presence of well-marked goitre in approximately 2.5 per cent of school children, these goitres being unrelated in their origin to endemic influences.

In Himalayan regions where the classical type of endemic goitre exists alone the iodine content of the soil is in general less than 5 parts per 10 millions although it may sometimes range between 10 and 45 parts per 10 million parts of the soil But the disease is not always endemic in villages whose soils are as poor in iodine as those of other villages in which the endemic prevails

There is no evidence that in the Himalayan India the incidence of the disease is in inverse ratio to the iodine content of the soil In the heart of the endemic zone as well as in the Himalayan foot hills two places adjacent to one another may have approximately the same amount of iodine in their soils yet goitre be endemic in the one and not in the other, or the soils of two adjacent villages may be equally poor in iodine yet goitre be four times as prevalent in the one as in the other although the water supply and conditions of life of the inhabitants are the same

The water supply of one place may contain appreciable amounts of iodine (300 parts per 100 billion parts of water) and yet goitre may be endemic therein the water supply of another and adjacent place may contain no determinable amount of iodine and yet goitre may not be endemic therein, the water supply of two places some miles apart may be the same and yet goitre be present in the one and not in the other

Drinking water containing 300 parts per 100 billion parts of water has not prevented the occurrence of the disease in endemic form in the presence of a high degree of bacteriological impurity of the water nor has the consumption of a drinking water containing 1 200 parts of iodine per 100 billions prevented the development of goitre under experimental conditions in young men who consumed daily large amounts of the unboiled suspended matter removed by filtration or by sedimentation from a grossly polluted goitre producing water

The substitution of a bacteriologically pure for a bacteriologically impure water has caused the rapid and complete disappearance of the disease from a place in which it had been endemic for 70 years although the new water supply contained less iodine than the old

Iodine containing salts or substitutes for salt appear to have an influence in preventing this type of endemic goitre

The disease is in general more prone to arise in iodine poor than in iodine rich localities in Himalayan India though iodine poor localities both in the hills and in the plains may be free from it

Iodine deficiency is not the essential cause of the endemic goitre which prevails in Himalayan India though this deficiency is favourable to its development The essential cause of the disease is the presence in the body of toxic metabolites arising in consequence of bacterial action in the intestinal tract

DISCUSSION

Dr T S Tirumurti (Madras) Col McCarrison has given a separate name to the goitre experimentally produced by him He calls it a lymph adenoid goitre

Histologically it is characterized by excessive infiltration of round cell lymphocytes In later stages there is marked fibrosis We know these are the characteristics of all chronic inflammations I desire to know whether he is not describing acute and chronic inflammatory conditions in the thyroid gland It appears to me unnecessary to give the special name—lymph adenoid—to this type of goitre I wish to know whether these lymph adenoid goitres are of inflammatory origin

Lieut Col. R McCarrison, I M S (B India) replied The term lymph adenoid goitre ' is one used by Drs Williamson and Pearse who originally described the condition in man It is the hypertrophic reaction of a physiologically inefficient organ—no doubt to toxic or bacterial excitants—with which is associated a preponderance of lymphocytic aggregates and fibrosis in the gland

IMMUNOLOGY AND CHEMOTHERAPY

ON SOME FACTORS INFLUENCING THE THERAPEUTIC VALUE OF THE SOLUTIONS OF SALVARSANS

BY

SAHACHIRO HATA
Keio Gijuku University Medical College, Kitasato Institute, Tokio

DNESDAY, 7TH A.M. TO M.

IN the practical application of the solutions of salvarsans there have been increasing tendencies to employ various substances either in mixture with salvarsans or simultaneously for various purposes. It is however regretted that little fundamental knowledge concerning the influences of these substances upon the toxicity or therapeutic value of salvarsans are available. Dr. T. Komatsu of my laboratory has been experimenting for a long while with small animals whether or not the widely employed substances influence the practical value of salvarsan solutions. From among his wide sphere of studies I am going to-day to touch upon two facts only which have been observed by him. They might appear to be of a minor importance or even indifferent in the therapeutic value of salvarsan solutions, but you will marvel at the influences they have upon the value of salvarsans.

I. REACTION OF SOLVENT

It is well known that salvarsan has an acid reaction. In the practical application it is first to be alkalized as it is stated in the directions for use and then to be diluted with the saline. Neosalvarsan is generally considered to be neutral. In fact, however, some batches are neutral, many are slightly alkaline, while we seldom find the ones having a faintly acid reaction. Usually they are employed by being dissolved either in the distilled water or saline solution. If 'old' salvarsan should be dissolved with just sufficient amount of alkali to effect the complete dissolution and then diluted with the saline to a high degree, turbidity would be observed to develop. To avoid this inconvenience the use of a little excess amount of alkali is given in the directions. But in the case of therapeutic experiments of trypanosomiasis which will be dealt with later the solution must be highly diluted and it therefore appears somewhat turbid. In this case a slightly alkaline solvent is necessarily to be employed. We carried out experiments to determine the influences of the reaction of the solvent upon

the toxicity and the therapeutic value of salvarsan solutions by employing the solvents having different reactions In the experiments we employed six batches of salvarsan and twenty eight of neosalvarsan both of the Japanese and the German made and the following two solvents One was a 0 6 per cent saline made of freshly distilled water If left standing in air it will become slightly acid by absorbing carbon dioxide Therefore it must be freshly prepared just before the use This solvent is called for convenience's sake the 'neutral solvent' The other was the neutral solvent mixed with a certain amount of caustic soda to make up about a 1/1000 normal solution This is named the 'alkaline solvent' The differences in the toxicity and the therapeutic value of salvarsan solutions produced by the use of these two solvents will be seen in the following experiment —

The method of our experiment agreed on the whole with that recommended by the standardization committee of certain drugs on the health section of the League of Nations as the international method

The experimental animals were mice having the body weight ranging between 15 – 20 grammes The estimation of toxicity was made by injecting slowly into the tail vein 1 0 c c of the solutions of different concentrations per 20 grammes of the body weight *five or more mice being employed for each* dilution The highest concentration with which more than 60 per cent of the injected animals survived even the period of fortnight's observation is said to be the *dosis tolerata maxima* of the given specimen As the test objects for the therapeutic experiments the mice were infected with either *Spirochaeta duttoni* or *Trypanosoma gambiense* The infection was made in such a degree that 1 – 3 parasites would be found in every 20 microscopic fields of the fresh blood specimen under the dark field illumination on the 20th hour after the infection when salvarsans were intravenously injected in the amount of 1 0 c c per 20 grammes body weight in different concentrations The examination of the blood was made once every day for a fortnight and the lowest concentration which could make even more than 50 per cent of the treated mice free from the parasites during that period of time is titred as the *dosis curativa minima*

TABLE I

Toxicity of Salvarsan Solutions Having Different Reactions

Doses		I			II		
	Solvents	Number of mice	Number of survivors	Percentage	Number of mice	Number of survivors	Percentage
Neosalvarsan	Neutral	95	74	77 9	80	45	56 0
	Alkaline	80	54	67 5	70	32	45 7
Salvarsan	Neutral	30	23	76 7	30	14	46 7
	Alkaline	30	20	66 7	30	13	43 3

In Table I all the results of the tests for the toxicity of the solutions of various batches of salvarsans are summarized As the *dosis tolerata maxima*

of each different salvarsan preparation is different and as it is difficult to show the figure with each individual batch in the Table, I summarized the results into two groups concerning doses 'I' in the table represents the *dosis tolerata* of the batches in neutral solvent, while 'II' about 20 per cent more concentrated than I The results are that both salvarsan and neosalvarsan dissolved in the alkaline solvent have higher toxicity than in the neutral

TABLE II

Therapeutic Action of Neosalvarsan Solutions Having Different Reactions

Test object	Preparation	Neutral Solvent			Alkaline Solvent	
Trypanosoma gambiense		Doses	1 12 000	1 9 000	1 3 000	1 1000
	Neosalvarsan V N J J		2-3	3 3	0-3	3 3
	Neoarsaminol N 1110		2 3	3 3	0 3	3 3
	Normal Neotansalvan AKI		1 3	3 3	1 3	1 3
Spirochaeta duttoni		Doses	1 350	1 300	1 300	1 250
	Neosalvarsan EJDN		0 3	3 3	0 3	1 3
	Neoarsaminol N 1032		1 3	2 2	0 3	1 3

Table II shows the results of the therapeutic experiments The denominators of the fractions entered in the table stand for the number of the tested animals while the nominators for the number of the cured ones From this table it will be clearly seen that there were differences in the therapeutic efficacy of salvarsans dissolved in the two solvents The parasiticidal action of neosalvarsan in the alkaline solvent against the trypanosoma as well as the spirochaeta was markedly weaker than in the neutral This is a new fact untouched by the previous investigators Therefore we carried out repeatedly the similar series of experiments Moreover we asked the manufacturer to prepare five different specimens of neosalvarsan having the reactions varying from neutral up to a certain degree of alkalinity and experimented with them The results were always the same i e the higher the alkalinity was the higher the toxicity, and the lower the therapeutic value The following experiment will demonstrate more evidently the influence of alkali upon the therapeutic value of neosalvarsans Mice were inoculated with trypanosoma and on the third day, when there appeared quite numerous parasites in the blood the treatment with a 1 500th solution of neosalvarsan in the two solvents was applied and it was observed how long it would take to effect the complete sterilization of the blood (*see* Table III)

TABLE III

Therapeutic Treat[illegible] of F[illegible] Advanced Experi[illegible]tal Trypanoso[illegible]asis (Mouse) with Neosalvarsan

Solvents	Neutral						Alkaline					Control		
Number of mouse	1	[illegible]	4	[illegible]	8	9	10	1[illegible]	13	14	17	3	[illegible]	1[illegible]
Before treatment	+++	+++	+++	++	+++	+ +	+++	+++	++	+++	++	[illegible]	++	+++
½ hour after treatment	++	++	+ +	+ +	+++	++	+++	+	+++	+++	+ +	Succumbed [illegible] hours after	Succumbed in the night	Succumbed [illegible] th[illegible] [illegible]t
[illegible] ,,	++	++	++	+	++	+		+		++				
1 ,, ,,		10	[illegible]	15	1	0	[illegible]			+				
1½ hours	0-3	1 3	3	1[illegible]	5-7	[illegible]	[illegible]-7	1	1		1			
2 ,,	[illegible]	0-1	0-	3-5	1-1	1-3	3 [illegible]	3 5	1 3	0	5 7			
2½		1 [illegible]	1 [illegible]	0-1	1 [illegible]	1 [illegible]	1 [illegible]	0	1	1[illegible]	3			
3				1 [illegible]			[illegible] 0	1	1	1	1-			
3½ ,,							1 [illegible]	1	1	[illegible]	0-1			
4								1		1 [illegible]	1 [illegible]			
5 ,,														

Number of tryp[illegible]

N[illegible]e.—The symbols + a[illegible] figures are the n[illegible] of the spe[illegible] [illegible] trypano[illegible] [illegible] dark [illegible] They are as follows: +++ stands for about 30 [illegible] [illegible] [illegible] 3 a[illegible] the [illegible] the [illegible] [illegible] from [illegible]

TABLE IV.

Therapeutic Experiments with Salvarsan Solutions Having Different Reactions

Test object	SOLVENTS	NEUTRAL.		ALKALINE	
	Dilution	1 12,000	1 10 000	1 15,000	1 12,000
T. gambiense	Arsaminol 160	1–3	3–3	1–3	3–3
	Dilution	1 1,200	1 1,000	1 1,500	1 1,200
S duttoni	Arsaminol 160	1–3	2–3	2–3	3–3

TABLE V.

Summarized Table Showing the Differences of the Therapeutic Values of Salvarsan Solutions Having Different Reactions

Test object	SOLVENTS	NEOSALVARSAN			SALVARSAN.		
		Number of mice	Number cured	Percentage	Number of mice	Number cured	Percentage
T gambiense	Neutral	86	70	81 4	60	29	48 3
	Alkaline	83	0	0	54	35	64 8

Salvarsan gave just the reverse results, i e , it had a higher therapeutic value in the alkaline solvent than in the neutral (*see* Table IV) The difference of the value was especially remarkable with trypanosomiasis The summarized results of a great number of series of experiments, which have been made with a number of batches are shown in Table V From this table, it will be seen that if the

concentration of salvarsan should be the same in both solvents and taking the amount of salvarsan just equal to the *dosis curativa* in the neutral solvents the alkaline solution of neosalvarsan had less value than the neutral but with the old salvarsan, the alkaline solution had rather higher value than the neutral The salvarsan committee of the British Medical Research Council carried out investigations into the by effects of salvarsans and recommended that for dissolving of salvarsans an ample amount or rather excess of alkali should be employed This I presumably consider was intended to provide against the untoward effects arising from the use of an insufficient amount of alkali but I have reasons to believe that I cannot so readily consent to the use of an excessive amount of alkali as to the insufficiency of it From our experiments we discovered the fact that too much alkali resulted in increasing the toxicity I might be justified here in adding that in the experimental treatment of trypanosomiasis a very high dilution of salvarsan solutions is required but if in this kind of experiment salvarsans should be attempted to be diluted to the desired magnitude in neutral solvent the liquid would become somewhat opaque and its therapeutic action would be decreased In the treatment of human cases however such a high dilution as in the treatment of experimental trypanosomiasis is never employed and it is needless to employ an excess amount of alkali

II Concentration of Salvarsan Solutions

Since the very beginning of my studies on salvarsan I have been aware of the fact that the concentration of the solution does indeed influence the toxicity When injected intravenously a small volume of a certain concentration of salvarsan was more toxic than a larger volume of the solution containing the same amount of salvarsan as the former For instance a rabbit having the body weight of two kilograms fairly tolerates 0 2 grm of salvarsan dissolved in 50 0 c cs (or a 1 250 solution) while the same amount of salvarsan in a 10 0 c cs (or a 1 50 solution) sometimes kills the animal This has since been confirmed by several investigators It is considered necessary to investigate very closely with neosalvarsan which is usually employed in a very high concentration The influences of the concentration of salvarsan upon the therapeutic value has been reported by Kroo and Mano(1) while our experiments were being carried on Taking their work also into our consideration we studied with many batches of salvarsan and neosalvarsan the relation of the concentration to their toxicity as well as to their therapeutic value

In our experiments increasing files of concentration were employed In this communication however I will only deal with the results we obtained with two different concentrations One series consists of such a degree of concentration as a certain dosis of salvarsans intended for a 20 grm mouse to be contained in 1 0 c c of the solution while the other series contains the same dosis in 0 1 c c of the solution One is called the "weak" solution while the other the "concentrated"

solution The method we employed for the determination of the *dosis tolerata* and *curativa* were the same as I have stated before The only difference, however was that in the injection of the concentrated solution, owing to the small bulk of the solution we employed an especially finely graded slender and long syringe manufactured for this especial purpose Again in order to inject the whole dosis just within the same length of time as the weak solution the injection of the concentrated solution was made far slower than that of the weak solution

TABLE VI

Summarized Table Showing the Differences of Toxicity caused by the Differences of Concentration of Salvarsan Solutions

	Doses	I			II		
	Concentration	Number of mice	Number of survivors	Percentage	Number of mice	Number of survivors	Percentage
Neosalvarsan	Weak	9[illegible]	74	77 8	8[illegible]	4[illegible]	[illegible]2 9
	Concentrated	14[illegible]	[illegible]	5[illegible] 6	14[illegible]	50	3[illegible] 2
Salvarsan	Weak	30	26	86 7	30	19	63 3
	Concentrated	30	15	50 0	30	9	30 0

The oscillation of the toxicity according to the difference of the concentration will be seen from Table VI In this table comparative results of the two different groups of doses are shown 'Dosis I' is meant to represent the use of *the dosis tolerata* of the weak solution while the 'Dosis II' the use of the dosis about 20 per cent larger than the former because the *dosis tolerata* of each individual batch of preparations differs from each other and it is impossible to show *their figure of each individual batch* With all the cases the weak solution had a larger percentage of survivors than the same dosis in the concentrated solution This difference of the toxicity arising from the differences in the concentrations was far more remarkable with salvarsan than with neosalvarsan

Table VII

Differences of Therapeutic Value caused by Differences of Concentration of Salvarsan Solutions

Parasites	Preparation.	Amount per 20 grm	1 0 c c			0·1 c c		
		Concentration	1 12 000	1 10 000	1 8 000	1 1 200	1 1 000	1 800
T gambiense	Neosalvarsan FDHN		4-5	4-5		0-5	0-5	
	Neoarsaminol N 1113		5-5	5-5		0-5	0-5	
	Arsaminol 161		1-3	2-3	3-3	0-7	1-3	1-3
		Concentration	1 350	1 300		1 37	1 30	1 25
S duttoni	Neosalvarsan NNBN		2-5	5-5		0-4	1-4	2-4
	Neoarsaminol N 1112		3-5	5-5		0-3	0-3	2 3
		Concentration	1 1 000	1 800	1 600	1 100	1 80	1 60
	Salvarsan MMJN		1-4	3 3	3 3	0-4	1 4	4-4
	Arsaminol 161		1-4	2-4	4-4	0-4	0-4	2-4

In Table VII a few examples of the influences of the concentrations upon the therapeutic value of salvarsans are shown Both the weak and the concentrated solutions of several batches of salvarsans were employed for the treatment of trypanosomiasis and relapsing fever In all the cases the weak solution had a higher percentage of cure than with the concentrated one This difference was by far the more remarkable with neosalvarsan than with salvarsan Again

prepared with such a precaution as to be as strictly neutral as possible The tendency to supply the solvent, *i e*, distilled water, in hermetically sealed vials is recently very widely growing A long kept distilled water even sealed in a glass vial has often been found to become slightly alkaline, and therefore the vial should be made strictly of hard glass

Secondly The fact that the higher the concentration of salvarsan is the more disagreeable effects it brings both upon the toxicity and the therapeutic value is the factor which should require a deep consideration on the side of the practical clinicians In the early period of salvarsan therapy, Ehrlich and I recommended the use of a highly diluted solution, but since the introduction of the problem of 'water failure' the tendency of using a highly concentrated solution has been more and more influential Especially this tendency has been supported by the introduction of neosalvarsan, which has nothing of the direct ill effects even when used in a highly concentrated solution and more by the simplicity of the technique Thus the use of a highly concentrated solution has now become almost routine in practice I am not venturing to conclude that the results obtained in our experiments upon the small animals can be applicable as such to the clinical treatment of human cases but being affirmed of the facts that in our animal experiments the influence of the concentration was observed to occur in a similar manner with relapsing fever and trypanosomiasis in spite of the fact that in these two cases the concentration of the solution widely differed from each other I should like to consider that similar influence of the concentration should be met with in the treatment of human cases also I desire, therefore that this point should be still further studied on a very large number of patients It will be needless here to add that the use of a highly diluted solution is effective to check the development of disagreeable by effects

REFERENCE.

(1) Kroo and Mayo 19[illegible]) *Deutsch Med Woch* No. 15

THE RELATION BETWEEN THE CHEMICAL CONSTITUTION OF ANTIMONIALS AND THEIR THERAPEUTIC PROPERTIES

BY

RAI BAHADUR U N BRAHMACHARI, M A, M D, Ph D,
Calcutta

IN this paper the relationship of the chemical constitution of the following antimonials to their therapeutic properties are discussed —

(1) Metallic antimony
(2) Antimony trioxide
(3) The antimonyl tartrates and malates
(4) Acetyl p amino phenyl stibinate of sodium
(5) P amino phenyl stibinate of sodium
(6) Urea para stibanilate
(7) Ammonium carbamino stibanilate (Urea stibamine)
(8) N phenyl glycine amide p stibinate of sodium

Antimony belongs to the odd series of group V of Mendelieff s periodic system of elements in which the gradual transition from typical non metals to typical metals is clearly exhibited Phosphorus is decidedly a non metal while antimony and bismuth are typical metals although they are brittle Arsenic which stands between these two classes shows properties belonging to both groups of elements *The acid producing properties of antimony are greater than those of bismuth* and less than those of arsenic It has the property of combining with tartaric acid and giving rise to an acid of the type of what has been termed antimonyl tartaric acid Tartar emetic and sodium antimonyl tartrate should not be regarded as antimony salts of an organic acid They are really potassium and sodium salts of antimonyl tartaric acid as has been proved by Clarke Stallo Jungfleisch Guntz Adam and others Antimony exists in them not as a basic Sb=0 in combination with tartaric acid but as ortho antimonious acid Sb $(OH)_3$ in which two of its hydroxyl groups are replaced by the divalent group $C_4H_4O_6$ giving rise to antimonyl tartaric acid In other words antimony in tartar emetic and allied compounds exists in an acidic state

The most important factor upon which the therapeutic value of an antimonial depends is its property of containing trivalent antimony in an acidic state or its ability of being converted into a compound of this kind after its introduction into the body which will further possess the mobility of being converted into a

compound containing the radicle $Sb=O$ in a reactive state or in a highly dispersed condition

The *reactive* state corresponds more or less to the *nascent* state of elements and *mobility* means the quickness with which the property referred to above is displayed

The antimonyl tartrates finely divided metallic antimony or an aromatic antimonial derived from stibanilic acid all more or less possess these qualities and the superiority of an antimonial over another depends upon the degree of its power of exhibiting them

In studying trypanosomiasis Ehrlich held the view that trypanosomes assimilated the organic derivatives of arsenic only when the arsenic was present in the trivalent and not in the pentavalent form Similarly the experiments of Kolle, Hartoch Pothermundt and Schurmann have shown that compounds containing pentavalent antimony were not organotropic except in large doses and were at the same time slightly parasitotropic Preparations containing trivalent antimony were, as a rule toxic to the organism and at the same time it was also shown by these observers that for antimony compounds soluble or insoluble organic or inorganic, to be of therapeutic value in trypanosomiasis the antimony must be in the trivalent form My most recent researches and those of others that have followed me have however proved that the aromatic pentavalent antimonials are much more potent in the treatment of kala azar than the trivalent antimonyl tartrates I hold that it is not so much whether an antimony compound is trivalent or pentavalent that is responsible for their therapeutic value but its capacity for being quickly converted into a compound containing $Sb=O$

By studying the excretion of antimony in man after intravenous injection of the aromatic antimonials of the type of urea stibamine and also of the antimonyl tartrates one can explain the superiority of the former over the latter on the above theory I have observed that in the case of tartar emetic the curve of excretion is one slowly converging to the base line

The amount of antimony excreted in the urine during the first 24 hours after intravenous injection of tartar emetic is about 6 per cent of the amount injected The amount of antimony excreted in the urine during the first 24 hours after intravenous injection of urea stibamine is 30 to 40 per cent of the amount injected The excretion of antimony after intravenous injection of a pentavalent organic antimonial follows a curve the first portion of which representing the excretion during the first 24 hours is abrupt and the second portion follows a course similar to that found in the case of tartar emetic It is probable that a pentavalent organic antimonial is converted in the body into a trivalent antimonial and that as long as it exists in the body in the pentavalent form its rate of excretion is much quicker than when it is converted into the trivalent form During the latter stage the curve of excretion is similar to that of tartar emetic in which antimony exists in the trivalent form Since a great portion of antimony present in an aromatic pentavalent antimonial (urea stibamine) is quickly eliminated the chances of toxic action of the compound

are much less than that of an antimonyl tartrate In the process of conversion of an aromatic pentavalent antimonial in the body into a compound containing trivalent antimony, a reactive Sb=O is *formed which is probably responsible for the* remarkably beneficial results observed in the treatment of leishmaniasis by the use of urea stibamine

Finely divided Metallic Antimony

Though various metals have been administered intravenously in the colloidal state metallic antimony is perhaps the only one which has been put into the circulation in the crude form of a fine suspension To Plimmer and Fry belongs the credit of first demonstrating the possibility of introducing metallic antimony into the veins without danger of capillary blocking To Ranken belongs the credit of *using the drug successfully in man in the treatment of trypanosomiasis by the* intravenous route

In 1915 I described a number of cases of kala azar treated successfully with intravenous injection of metallic antimony and I pointed out that it was the most powerful leishmanicide that was known at that time just as it was the most *powerful of the known antimonial trypanocides*

I observed that in cases in which the soluble salts of the type of tartar emetic did not show any improvement in the blood condition or temperature of the patient after several injections finely divided metallic antimony administered intravenously brought about complete cure In addition the number of injections required for a *course of treatment with metallic antimony was much smaller than those required* in the case of the antimonyl tartrates Three or four injections frequently cured the patient though sometimes the injections required were as many as eight or nine Even then the number of injections required for cure was less than what was generally required in the case of antimonyl tartrates The only objection is the complicated technique of the operation of injection which is a serious obstacle to mass treatment of the disease

The mechanism by which metallic antimony is taken up into the system after intravenous injections is very interesting *Quickly taken up by the leucocytes and* perhaps also by the cells of the reticulo endothelial system and without causing any capillary blocking it is converted into a soluble antimony compound as the particles of antimony sooner or later disappear from the leucocytes I consider that it is converted into a compound in which the antimony exists in a trivalent state and this conclusion has been arrived at by me by following the curve of excretion of antimony in the urine after its administration which resembles the curve of excretion of antimony after administration of tartar emetic

This trivalent antimony compound is subsequently converted into *one contain*ing a radicle of Sb=O in the reactive stage or in a highly dispersed condition It does not rest at the stage of a trivalent antimony compound allied to Sb_2O_3 because Sb_2O_3 when injected intravenously does not exhibit such therapeutic properties as

those of metallic antimony, as will be presently seen. It is possible that it is finally converted into nascent metallic antimony.

Levaditi has propounded a general law with reference to all the members of the nitrogen family of elements occupying Group V of Mendelieff's periodic table such as arsenic, antimony or bismuth. They or their compounds exhibit their parasiticidal properties only after they have been acted upon by the tissues. If fresh extract of liver is added to them, then they exert their parasiticidal properties.

I have observed that if a solution of tartar emetic or urea stibamine is mixed with a culture of leishmania and the mixture examined on a slide, the leishmania do not die. Like bismuth or its compounds they become active only after they have been acted upon by the tissues.

It has been suggested that in the case of bismuth the action of the cellular extract gives rise to a new compound 'bismoxyl' and it is this which possesses the destructive power against the *Treponema pallidum*. The substance in the extract which has the property of changing bismuth into bismoxyl has been termed 'bismogene'.

Bismoxyl is supposed to be a bismuth toxalbumin.

Chemically some of the bismuth compounds contain the radicle Bi=O, just as some of the antimony compounds contain the radicle Sb=O, and it is very likely that the bismuth toxalbumin also contains the radicle Bi=O in the reactive stage *or in a highly dispersed condition. A corresponding antimony compound which* may be called 'stiboxyl' is probably formed in the case of antimony.

It has been recently observed by Meleney that in kala azar clasmatocyte tissue is developed as a tissue reaction and probably, as I have suggested, out of the reticulo endothelial system. I hold that this reticulo endothelial system gives rise to the production of bismoxyl or stiboxyl at the case may be.

I have discussed in detail what I consider is the mechanism by which metallic antimony exerts its parasiticidal properties, because being a simple element it does not contain any groups of radicles which may complicate any explanation that may be suggested. The sequence of events in this mechanism may be summarized as follows:—

Metallic antimony—taken up by leucocytes and cells of the reticulo-endothelial system—a soluble trivalent antimony compound—an antimony compound containing Sb=O in the reactive stage or dispersed conditon (stiboxyl) or nascent antimony which acts as a leishmanicide in kala azar.

Colloidal Metallic Antimony

Colloidal metals are remarkable in having minimum organ tropic properties and at the same time are frequently parasitotropic. An ideal medicament should be one in which the ratio of *dosis curativa* to *dosis tolerata* should be as low as possible. Because of the extreme division of the metals in the colloidal state there is an immense surface of contact between a colloidal solution or suspension and the surrounding medium. For instance, it has been calculated that the total surface of

d in one cubic centimetre of colloidal gold may attain to nearly 6 500
This immense contact surface of the colloidal suspension of metals,
harge of constant sign *for the same substance and the fact that in the*
reactions that take place are nearly always between colloids, render
es of metallic colloids very great

Sb_2O_3—Antimony Trioxide

Blackmore have used trixidine in oily suspension intramuscularly
used a fine preparation of the same intravenously
toch Rothermundt and Schurmann consider that the formation of a
nsoluble slowly absorbable compound of antimony, such as antimony
prophylactically against trypanosome infection The principle of
it of insoluble organic compounds of antimony, *either in ointment*
the formation of intramuscular depots, constitutes, what the authors
na mite curans as contrasted with *therapia magna sterilans* Rogers
azar In my experience it is weak in its therapeutic properties in

try to explain why antimony trioxide is feebler in its leishmanicidal
metallic antimony Though antimony exists in it in the trivalent
lity of being converted into a compound containing Sb=O in a reactive
as it is a fairly stable compound This theory agrees with the
more potent than Sb_2O_5 which is more stable and has much less
properties Of all the oxides of antimony, Sb_2O_4 is the most stable
ware Sb_2O_4 has no use whatever in therapeutics One may, therefore,
general rule that the more stable is an oxide of antimony, the less is
l or leishmanicidal property

Tartar Emetic and other Antimonyl Tartrates

before these are salts of antimonyl tartaric acid and have been
nsidered *as antimony compounds of tartaric acid even in a recent*
a azar
antimonyl tartrates of the type of tartar emetic or sodium antimonyl
ed ammonium antimonyl tartrate, urea antimonyl tartrate, aniline
ate, ethyl antimonyl tartrate quinine antimonyl tartrate, cinchonine
arcotine antimonyl tartrate
T (Urea) T (K) T (Na) T (Aniline) etc , represent *the toxicity of*
ates respectively, *I have observed* —

$$\frac{\text{H}_4)}{\text{ea})} = \frac{\text{T (NH}_4)}{\text{T (K)}} = \frac{\text{T (NH}_4)}{\text{T (Na)}} = \frac{\text{T (NH}_4)}{\text{T (Aniline)}} = \frac{65}{60} \text{ or } \frac{11}{12}$$

H$_4$) T Sb (Urea), T Sb (K) T Sb (Aniline) T Sb (Na) represent
he antimony content of the above tartrates, we have —

$$= \frac{41}{46}, \frac{\text{T Sb(NH}_4)}{\text{T Sb(K)}} = \frac{40}{46}, \frac{\text{T Sb(NH}_4)}{\text{T Sb(Na)}} = \frac{38}{46}, \frac{\text{T Sb(NH}_4)}{\text{T Sb(Aniline)}} = \frac{35}{46}$$

refore in the case of the guinea pigs ammonium antimonyl tartrate is the xic, then comes the urea salt, then the sodium and potassium salts which ally toxic and then the aniline salt

maximum tolerating capacity of the same species of animals for a drug is *proportional* to its maximum tolerated dose

thus have --

Maximum tolerating capacity of guinea pigs treated with ammonium antimonyl tartrate $= K^1 \times 03$

Do	Do	Do	Urea antimonyl tartrate $= K^1 \times 025$
Do	Do	Do	Potassium antimonyl tartrate $= K^1 \times 015$
Do	Do	Do	Sodium antimonyl tartrate $= K^1 \times 015$
Do	Do	Do	Aniline antimonyl tartrate $= K^1 \times 025$

this we conclude that of all the antimonyl tartrates used in the case of pigs their maximum tolerating capacity is with ammonium antimonyl nd that the presence of N in the basic radicle of an antimonyl tartrate the toxicity of some of them

ally speaking the toxicity of the antimonyl tartrates depends upon their content A notable exception is in the case of quinine antimonyl tartrate he toxicity is low The possibility of using the compound in therapeutics refore be borne in mind as it may combine the therapeutic properties of and quinine

e not however, been able to confirm the observations of Farghar and the toxicity of the antimony content of quinine antimonyl tartrate is ifth that of tartar emetic though I agree with them that its toxicity is less of tartar emetic I confirm their observations that quinine antimonyl n boiling with antimony trioxide is converted into the more toxic quino timonyl tartrate *I have not been able to confirm their* as well as Rogers', is that the sodium salt is less toxic than the potassium salt I have Plimmer and Thompson's observations that the lithium salt is more n the sodium or potassium salt and that the toxicity of sodium and salt is equal

e further found that ammonium antimonyl tartrate is the least toxic of organic tartrates the presence of nitrogen in the basic radicle diminishing ty Because of the high antimony content of the ammonium salt, its low toxicity for the lower animals and likewise for human beings and as found to possess a marked degree of therapeutic activity in the treat- kala azar, I consider it superior to both potassium and sodium antimonyl

I found that after the administration of a toxic dose of an antimonyl tartrate, the pathological changes are most marked in the lungs, kidneys, liver, pituitary and suprarenal glands, consisting chiefly of hæmorrhages into the substance of these organs and destruction of their cellular elements Similar changes were produced by toxic doses of new aromatic organic antimonyls

Delayed Antimony Poisoning —Cases of death in guinea pigs three weeks or so after one injection of an antimonial salt have been met with, showing definite symptoms of antimony poisoning and presence of antimony in the viscera

These cases of delayed antimony poisoning are of very great clinical importance, as they prove that the excretion of the drug may sometimes be very slow after injection of antimonial compounds and some of the cases of sudden death during antimonial treatment may be due to a cumulative action of the drug

Cumulative and Tolerance Experiments with Tartar Emetic —I have observed that repeated injections of tartar emetic in sub lethal doses did not give rise to any tolerance towards the drug except very rarely Generally the results pointed to a cumulative action of the drug, or at least made the animal susceptible to the next higher dose

We have observed before that antimony in tartar emetic and other antimonyl tartrates exists in the form of antimonious acid Sb $(OH)_3$ in which two hydroxyl groups have been replaced by the divalent $C_4H_4O_6$ When introduced into the system its therapeutic value depends upon its ability to give rise to a reactive—Sb=0—which theoretically speaking should be the same as that of the salts of hydrated Sb_2O_3 *i e*, $Sb_2O_3+3H_2O$ *or* $Sb(OH)_3$ *or ortho antimonious acid* Herein lies the superiority of the aromatic antimonials over the antimonyl tartrates which we shall presently see On the other hand, if it were possible to prepare an anti monial having the same composition as the antimonyl tartrates but having the radicle, Sb=0 as is shown in the old configuration of tartar emetic and allied salts, then such an isomer of tartar emetic would be more potent in the treatment of kala azar than tartar emetic itself We await the production of such an isomer

Besides the antimonyl tartrates already referred to, the following amino antimonyl tartrates have been prepared in my laboratory —

(1) Phenocoll antimonyl tartrate
(2) Anæsthesin antimonyl tartrate
(3) Novocaine antimonyl tartrate
(4) Aposthesine antimonyl tartrate
(5) Orthoform antimonyl tartrate
(6) Acriflavine antimonyl tartrate

The late Sir Patrick Manson once wrote to me as follows —

'Go on in your efforts to get an antimony compound that can be used as an intramuscular injection or, better still as a drug that can be administered by the mouth' The therapeutic value of an antimonial depends upon its concentration in the tissues after administration Unfortunately ordinary antimonials cannot be

administered orally intramuscularly or per rectum in such doses as to bring about his concentration without at the same time giving rise to local distressing ymptoms The above new amino antimonyl tartrates containing radicles possessing anæsthetic properties, may be worth trial by these routes

Ointment of metallic antimony in a state of finest sub division may be more easily absorbed and less irritating than that made with ordinary metallic antimony, and may be of therapeutic value in the treatment of kala azar

THE AROMATIC ANTIMONIALS

Let us now pass on to the consideration of the aromatic antimonials and their value in the chemotherapy of antimony

In 1920, shortly after I had been financed by the Indian Research Fund Association for carrying on researches into the treatment of kala azar I brought to the notice of the Government and the Governing Body of the Indian Research Fund Association, the possibility of the potentialities of organic antimonials in the treatment of Indian kala azar, my conclusions being based on theoretical grounds from an analogy of the value of the corresponding compounds of arsenic, namely *ars acetin* and *atoxyl*, in the treatment of certain protozoal diseases

The acetyl compound (stibacetin stibenyl) was used more or less successfully outside India in the treatment of kala azar and other forms of leishmaniasis (Caronia Kharina Marinuchi, Spagnolio) Manson Bahr successfully used it in a case of kala azar Early in 1921, I discovered that urea could combine with stibanilic acid and that the resulting compound surpassed all my expectations in its value in the treatment of kala azar The discovery of this compound and my researches into the chemotherapy of antimonial compounds in kala azar infection opened up a new vista in the treatment of the disease

The starting material of aromatic antimonials is acetyl p amino phenyl stibinic acid Theoretically speaking the value of the sodium salt of the acid in the treatment of kala azar should be the same as that of ars acetin in the treatment of trypanosome infection Ars acetin has certain marked advantages compared with atoxyl, being more stable and less toxic to some animals, while equally toxic to the parasites This diminution of toxic effect is, however noticeable only in certain animal species and not with horses or guinea pigs Voegtlin and Smith have observed that it is considerably less toxic than atoxyl and more trypanocidal, possessing a chemotherapeutic index about five times higher than atoxyl

It is a well-known theory in the case of aromatic arsenicals that their therapeutic value depends upon the reduction products produced after their introduction into the system These reduction products probably all contain the reactive As=O The trivalent aromatic arsenicals of the arseno benzene group possess the property of producing these reduction products to a greater extent than the pentavalent arsenicals and hence their superiority in the treatment of treponema and trypanosome infections over the pentavalent arsenicals, except

tryparsamide I hold that the therapeutic value of the aromatic antimonials also depends upon the same property

The comparative value of the aromatic antimonials in the treatment of kala azar also depends upon their toxicity and parasitotropic properties following their administration These again depend upon their chemical configuration and physico chemical properties In order that they may be of therapeutic use the ratio of their *dosis curativa* to that of their *dosis tolerata* must conform to Ehrlich s formula which is 1 3 or less

The Aromatic Antimonials of the Stibino Benzene Group

Antimonials of the stibino benzene type have not yet come into use in the treatment of human diseases though they have been used with indefinite results in the case of certain diseases of animals

Trivalent aromatic antimonials of the type of salvarsan or neo salvarsan will probably be in future the highest advance in the antimony treatment of kala azar

The Aromatic Antimonials derived from P arsanilic Acid (p amino phenyl stibinic acid)

The minimum lethal dose of phenyl stibinate of sodium is three and half times less than that of acetyl p amino phenyl stibinate of sodium while its maximum tolerated dose is 35 times less Injected into lower animals it gives rise to *hæmorrhagic nephritis and other symptoms of severe antimony poisoning* This compound has little or no use in therapeutics but the introduction of NH_2 into its benzene nucleus at once diminishes its toxicity and raises its therapeutic value to a remarkable extent Acetyl para amino phenyl stibinate of sodium (Stibenyl Stibacetin Sodium acetyl p stibanilate)

I have just now stated that ars acetin possesses certain special advantages over atoxyl Unfortunately however it has not come so much into use as atoxyl or soamin The acetyl compound of antimony has been used in the treatment of kala azar but with unsatisfactory results *Besides as has been shown by me* stibenyl becomes toxic with age in India and it has now come into disuse But I still hold that pure acetyl p amino phenyl stibinate of sodium should again be *given a trial in* kala azar and may in future be found to be free from all those toxic effects that were exhibited by stibenyl

The sodium salt *formed* after hydrolysis of acetyl compound corresponds to atoxyl or soamin and is sodium p stibanilate Comparing its toxicity with that of [illegible] to

Thus while in the case of ars acetin the toxicity is marked[illegible] the introduction of the acetyl group into atoxyl being 1 5th that of atoxyl in the case of sodium stibanilate and the acetyl compound my observations have

shown that their toxicity is the same The M L D is 0 7 grm per kilo of body weight and the M T D is 0 35 grm per kilo of body weight in guinea pigs given intramuscularly in the case of both the compounds

The pure salt is fairly stable It has been stated by some observers in India that the compound is very easily decomposed Evidently the substance that they were using was impure or not properly prepared Three cases have been treated by me with this compound with satisfactory results But as the number of cases was so limited no attempt can at present be made to give a comparative estimate of the therapeutic values of sodium p stibanilate and urea stibamine—a compound to be discussed later on

Chloro Stibacetin (von Heydon 471) or Stibosan

This is a compound formed by the replacement of one hydrogen atom in the benzene nucleus of the acetyl compound by chlorine The published results of cases treated with this compound lead to the conclusion that it is weaker in its therapeutic effects when compared with urea stibamine It has been claimed that the introduction of chlorine increases its stability It has also been claimed that it can be stored in ordinary stoppered bottles and weighed out when required and is therefore most useful for general purposes In my opinion such a compound has more or less the same stability as the antimonates and therefore there is less chance of the production of the reactive Sb—0 in the tissues after their administration which I consider is responsible for the beneficial results following the administration of an antimony compound This explains why antimonates in which antimony exists in a pentavalent form are of very little use in therapeutics as they are very stable and quickly excreted unchanged after administration The same also holds good in the case of arsenic

The next aromatic antimonial discovered by me is urea stibamine I shall discuss its therapeutic value later on

The next aromatic antimonial of probable therapeutic value that has been discovered by me is benzene sulphon p amino phenyl stibinate of sodium The corresponding arsenic compound is known as hectine which possesses certain therapeutic properties in syphilis The entrance however of a sulphonic group in the molecule reducing its toxicity also reduces its therapeutic properties and this fact is in accordance with the general physiological inertia of the sulphonic acids

Sodium allyl thiocarbamino p stibanilate is another compound of probable therapeutic value which has been produced in my laboratory The introduction of thio urea may reduce the toxicity of the compound just as it has been claimed in the case of the corresponding arsenic compound

Glucose Derivatives

The therapeutic value of the glucose compounds of the organic aromatic antimonials as compared with compounds from which they are derived is proportional to their antimony content and the same conclusion is arrived at on theoretical

considerations Their antimony contents are less than corresponding aryl antimonials from which they are derived and therefore a bigger dose has to be administered to be of equal therapeutic value The combination with glucose has therefore no advantage

The antimony content of some of the aromatic antimonials is given below —

Sodium stibanilate	42 10	per cent
Urea stibamine	36 95	
Chloro stib acetin (Stibosan)	33 30	"
Glucose sodium stibanilate	25 80	
Glucose urea stibamine	23 80	
N phenyl glycine amide p-stibinate of sodium	29 30	
Neo stibosan of unknown composition	42	

It may be stated that generally speaking the therapeutic value of the aromatic antimonials derived from p amino phenyl stibinic acid is proportional to their antimony content

As regards toxicity I have observed that the toxicity of pentavalent *compounds obtained from para stibinic acid is proportional to their antimony* content My observations are different from what I find stated in a recent book on kala azar from which it will be seen that urea stibamine and its glucose derivatives are regarded to be equally toxic The latter observation is rather significant as this would mean that the antimony content of the former is one and a half times less toxic than its glucose derivative

It is a well known fact that the sodium salt of N phenyl p arsenic acid is a substance of practically no importance in the treatment of experimental infections such as those produced in laboratory animals by various species of trypanosomes the spirochætes of relapsing fever and *Treponema pallidum* On the other hand N phenyl glycine amide p arsenate of sodium which is known under the trade name *of tryparsamide is the most effective arsenical yet produced for the treatment of* human sleeping sickness It has been stated that fruitful as Ehrlich s reduction theory proved in producing results of highly practical value it does not represent the whole truth for in tryparsamide arsenic exists in the pentavalent form But I hold that if a pentavalent organic arsenical when introduced in the system is more quickly converted into a compound containing the reactive As=0 than arseno benzene compounds then the former will be of more therapeutic value than the latter and on this the value of tryparsamide can be explained on Ehrlich s reduction theory

The most important and the last antimony compound that I shall now discuss is *urea stibamine* It is very much allied to tryparsamide

These compounds contain the group NH_2 CO and this to my mind is responsible for the therapeutic value of tryparasamide That being the case one would expect to have the same remarkably beneficial effects with antimony compounds containing this group in the treatment of diseases in

hich antimony is indicated just as tryparsamide in the case of human ypanosomiasis

This theoretical conclusion is borne out in practical experience For to day ea stibamine stands as the most pre eminent compound of antimony in the eatment of kala azar

Taking the published kala azar cases of different observers under different onditions and in different places treated with the aromatic antimonials the most xtended trial has been given to urea stibamine Observations upon the other romatic antimonials are mostly limited to observations of single individuals

In a combined series of 325 published cases which were treated by myself hortt Greig Kundu and others with this compound 98 47 per cent of the cases ere cured One of the cases died of extreme asthenia being admitted at the age f 65 in a moribund condition In 298 of these cases proof of cure was microscopic nd cultural examinations and disappearance of symptoms and in 27 cases proof f cure was clinical disappearance of the symptoms and subsequent observations f the cases One case was resistant

Regarding the value of N phenyl glycine amide p stibinate of sodium in he treatment of kala azar I have published a series of eight cases in which it was successfully used but no comparison can be made at present with urea stibamine as it has not yet been given an extended trial in the treatment of the disease

It has been proved by the observations of Shortt as well as those of myself and others that urea stibamine does not manifest any deterioration or other changes either in physical and chemical characters or in therapeutic properties if kept in sealed ampoules under ordinary conditions A more stable compound is undesirable as it will be less effective

I have already referred to my views of the reticulo endothelial system and I hold that individual cases will get beneficial results from the use of antimony compounds proportional to the reaction of the reticulo endothelial system Two things are necessary namely the development of the clasmatocytes and the intro duction of an antimony compound with which they can combine for the develop ment of stiboxyl Herein lies the value of the different antimonials and the superiority of urea stibamine over the other antimony compounds This also explains why with the same antimony compound one individual is cured much more quickly than another after its administration It is the response of the cells of the reticulo endothelial system to a particular drug that one should aim at in the treatment of the resistant cases

Let me now briefly refer to the views of Voegtlin and his co workers These observers have pointed out that arsenious oxide and its derivatives combine with substances containing a sulphydrile grouping and that the toxic action of the organic arsenoxides is depressed by the simultaneous injection of excess of sulphydrile compounds Hopkins has shown that one such sulphydrile com pound reduced glutathione plays an important part in the hydrolytic oxidation reduction processes of the living cell Voegtlin suggests that a combination of

the arsenoxides with such groups and consequent suppression of this vital function may explain the toxic and curative actions of the arsenical derivatives and that a formation by trypanosomes of the sulphydrile compound in excess of its vital need may be the basis of acquired resistance of trypanosomes The same probably takes place in the cases of *Leishmania* Investigations in these directions may lead to discovery of methods of preventing the development of antimony resistant *Leishmania*

We have discussed the chemotherapy of antimony from a certain standpoint based chiefly upon the ideas of Ehrlich Voegtlin and others Other factors that have to be considered in this connection are the molecular weight of the compounds their solubility their dissociation in solution their surface tension the hydrogen ion concentration of the tissue at which they act and various other points which I am afraid the time at my disposal will not permit me to discuss

I shall end here by quoting the remarks of Shortt and Sen which they made in 1925 about urea stibamine We consider the value of urea stibamine has been established as the most efficient drug at present in use in the treatment of kala azar' This statement remains equally true to day To this may be added the remarks of Dodds Price I am of opinion that urea stibamine is a most valuable remedy in the resistant types of the disease and I strongly urge that it should be resorted to if after a few injections of sodium antimonyl tartrate a patient does not show marked improvement I would only add that metallic antimony in a state of fine sub division *should be resorted to in those very few* cases which may be resistant to urea stibamine and which perhaps do not go beyond 3 per thousand or less

It will be seen from what I stated that Frankel in his 'Agrentimettel' is *not justified in saying* that changes in the molecular structure of antimony compounds do not bring about an increase in their therapeutic properties

CHEMOTHERAPY OF BUBONIC PLAGUE

BY

REV FATHER J F CAIUS, S J, F L S, M S C I (Paris)

AND

B P B NAIDU, M D (Edin), M H, D T M, D P H (L'pool),
Haffkine Institute, Parel, Bombay

THE discovery of salvarsan and the triumphs of chemotherapy in the treatment of diseases due to protozoa have stimulated investigation the object of which has been to find chemicals with a similar specific action in bacterial infections Much research work in bacterial chemotherapy has already been done in the past ten years, but to our knowledge, none which bears directly upon bubonic plague

This paper is a summary of the results we have obtained in a first attempt at (i) determining bactericidal action of antiseptics on *Bacillus pestis*, and (ii) ascertaining the effect of some of them on plague infected animals

I —BACTERICIDAL ACTION

To determine the comparative action of various antiseptics on *B pestis* we have adopted a technique which consists in cultivating the organisms in broth containing definite amounts of the antiseptic, and observing the exact concentration necessary to prevent development of the organism To this effect a 24 hours' plague culture in broth was used Five c cs of a solution of the drug were added to 5 c cs of the broth culture and the whole allowed to stand at room temperature (30° 31° C) for 15 minutes, 1 hour, 2 hours, 24 hours, 48 hours, when subcultures were made on agar slopes The tubes were incubated for three days at room temperature and the presence or absence of growth observed

Observation showed that under these conditions *B pestis* was actually destroyed so that the technique may be considered as measuring bactericidal power As a general result we have noted that though most drugs exert an appreciable germicidal action in 15 minutes, still their maximum efficacy is not attained until after a period of 24 hours We have recorded the results obtained after a contact of 15 minutes as they are of value in the selection of a germicide likely to be efficacious against *B pestis* and also the results obtained after a

contact of 24 hours as these furnish a reliable standard for the discussion of the correlation between chemical constitution and bactericidal power

We have employed in this enquiry solutions made up by dissolving the substance in just enough 5 6 per cent aqueous caustic soda and diluting to the required concentration *We had previously ascertained that the degree of alkalinity which is obtained in the various dilutions employed does not interfere with the growth of the plague organism* Failing this the sodium compound was prepared and its solution in water used

(1) *Phenols* —A comparatively high activity was manifested by the two isomeric dihydric phenols quinol (para dihydroxybenzene) and catechol (ortho dihydroxybenzene) The bactericidal value of quinol is greatly lowered by the entrance of a methyl group in its molecule (toluhydroquinone) The further entrance of an isopropyl group considerably depresses the bactericidal power and thymohydroquinone is more than 2 000 times less potent than quinol Etherification lowers the antiseptic value of the dihydric phenols

The trihydric phenols pyrogallol and phloroglucinol have a comparatively low bactericidal power

(2) *Substituted Phenols* —In general little change is brought about by substitution

The entrance of chlorine or bromine into the nucleus of phenol causes an increase in bactericidal power 2 4 6 trichlorophenol is sixteen times more powerful than phenol

The entrance of the amino group increases the bactericidal power 2 4 diaminophenol is twenty times as active as phenol, 2 amino 4 6 dinitrophenol (picraminic acid) is five times as active as 2 4 6 trinitrophenol (picric acid), 4 6 diaminoresorcinol is nearly four times as active as resorcinol

The entrance of the nitroso radicle in the molecule of betanaphthol makes this compound sixteen times more active

Substitution in the molecule of quinol leads to the formation of much less active substances

(3) *Mercurated Phenols* —The four compounds of this class result from the mercuration of phenol and are characterized by the presence of a nuclear hydroxyl and by the mercury being linked to the nuclear carbon through one valency and to either an atom of chlorine or to an acetoxy group through the other

The entrance of mercury in the nucleus of phenol considerably enhances its bactericidal value

(4) *Phthaleins* —*Phthaleins are compounds obtained by the condensation of two molecules of a phenol with one of phthalic anhydride* If phenols are condensed with the anhydride of orthosulphobenzoic acid instead of phthalic anhydride the compounds formed are analogous with the phthaleins and are known as sulphureins or sulphonephthaleins

As a class the phthaleins have a relatively small bactericidal value

(5) *Dye stuffs* —Resazurin obtained by oxidizing a mixture of nitroso resorcinol and resorcinol is an *oxazine* and belongs to the group of dye stuffs derived from quinoneimide It is nearly eight times as active as resorcinol

Fluorescein obtained by coupling *resorcinol with phthalic anhydride is the* phthalein of resorcinol and belongs to the group of dye stuffs derived from *xanthene* It is very little toxic to *B pestis* and so too are most of its derivatives the tetrabromo compound (eosine) in particular being inactive

Acid Fuchsin a *triphenyln ethane* dye is not toxic to *B pestis*

The two *benzidine* dyes trypan blue and trypan red have no action even in such a high concentration as 1 in 50

The two *acridine* dyes acriflavine and rivanol are slow acting but exert a marked germicidal action after 24 hours

(6) *Mercurated Dye stuffs* — The substances are derivatives in which the mercury is linked to the nucleus through one valency and either to a hydroxy or to an acetoxy group through the other They are many times more active than the compound they are derived from In general the bactericidal power is proportional to the amount of mercury present and but little change is brought about by modification in chemical constitution

That mercury dye stuffs owe their activity to the nuclear mercury is well illustrated by comparing the bactericidal powers of the following —

Resorcinol	1 800
Fluorescein (-resorcinol phthalein)	1 150
Monobromfluorescein	1 300
Mercurochrome (-dibromohydroxymercurifluorescein)	1 102 400
Tetrabromofluorescein	*Nil*

II Therapeutic Value

To find the bactericidal effect of a chemical compound *in vitro* is not so difficult but when it comes to experiments *in vivo* which are of particular importance in chemo therapeutic work the conditions become very complicated

The chemo therapeuticals studied were mercurochrome 220 soluble the diacetoxymercuric derivative of trypan blue and resorcinol

One of us (B P B N) (1) has already published the results obtained with mercurochrome and shown that the drug has no influence on the duration or termination of plague in rabbits and rats The same is the case with resorcinol and the mercurated trypan blue However we are not yet in a position to condemn chemotherapy in plague as utterly useless for our work is still in progress

REFERENCE

(1) Naidu B P B (1926 *Ind Jour Med Res* Vol XIV No 2 Oct.

Protocols

I – Monohydric Phenols

Compound	Bactericidal Concentrations	
	15 minutes	24 hours
phenol	1 600	1 600
o cresol	1 800	1 1 000
m-cresol	*1 3 600*	*1 3 600*
p cresol	1 2 400	1 [illegible] 400
isobutylphenol	1 [illegible] 800	1 4 800
isoamylphenol	1 3 [illegible]00	1 4 800
thymol	1 [illegible]00	1 300
carvacrol	*1 9 600*	*1 11 200*
alphanaphthol	1 1 [illegible]00	1 1 600
betanaphthol	1 1 200	1 2 400

II —Polyhydric Phenols

Compound	Bactericidal Concentrations	
	15 minutes	24 hours
A *Dihydric Phenols* —		
catechol	1 [illegible]4 000	1 48 000
resorcinol	1 800	1 800
quinol	1 308 000	1 432 000
toluhydroquinone	1 4 800	1 14 400
thymohydroquinone		1 [illegible]00
B *Trihydric Phenols* —		
pyrogallol	1 400	1 1 000
phloroglucinol	1 200	1 600

III —Ethers of Dihydric Phenols

Compound.	Bactericidal Concentrations	
	15 minutes	24 hours
catechol	1 24 000	1 48 000
guaiacol	1 400	1 800
resorcinol	1 800	1 800
resorcinmonomethylether	1 150	1 600
quinol	1 308 000	1 43[illegible] 000
hydroquinonemonomethylether	1 480	1 960
guaiacol	1 400	1 800
eugenol	1 2 000	1 4 000
isoeugenol	1 1 500	1 2 500

IV —Substituted Phenols

Compound	Bactericidal Concentrations	
	15 minutes	24 hours
phenol	1 600	1 600
2 4 6 trichlorphenol	1 2 000	1 10 000
2 4 6 tribromphenol	1 1 500	1 3 000
2 4 diaminoq henol	1 9 600	1 12 800
[illegible] 4 6 trin trophenol	1 250	1 400
[illegible] amino 4 6 dinitrophenol	1 600	1 2 000
resorcinol	1 800	1 800
4 6-d am noresorcinol	1 2 400	1 3 000
betanaphthol	1 1 200	1 2 400
alphan trosobetanaphthol	1 3 200	1 25 600

V —*Mercurated Phenols*

Compound	Bactericidal Concentrations	
	15 minutes	24 hours
phenol	1 600	1 600
2 4 6 trichlorphenol	1 2 000	1 10 000
o chloromercuriphenol	1 3 0 000	1 2 560 000
p chloromercuriphenol	1 320 000	1 5 120 000
o p dichloromercuriphenol	1 320 000	1 1 280 000
o p diacetoxymercuriphenol	1 160 000	1 640 000

VI —*Phthaleins and Sulphonephthaleins*

Compound	Bactericidal Concentrations	
	15 minutes	24 hours
phenol	1 600	1 600
phenolphthalein	1 200	1 200
phenolsulphonephthalein	1 800	1 1 200
o cresol	1 800	1 1 000
o cresolphthalein	1 1 200	1 1 600
o cresolsulphonephthalein	1 1 600	1 1 600
thymol	1 200	1 300
thymolphthalein	1 1 600	1 2 400
resorcinol	1 800	1 800
fluorescein	1 15	1 150
pyrogallol	1 400	1 1 000
gallein	1 100	1 200

VII.—Dye stuffs

Compound	Bactericidal Concentrations	
	15 minutes	24 hours
resazurin	1 4 800	1 6 000
fluorescein	1 150	1 150
acid fuchsin	no action	no action
trypan blue	no action	no action
trypan red	no action	no action
acriflavine	1 100	1 2 400
acriflavine neutral	no action	1 [illegible]56 000
rivanol	no action	1 12 800

VIII.—Mercurated Dye stuffs

Compound	Bactericidal Concentrations	
	15 minutes	24 hours
trypan blue	no action	no action
Hg trypan blue (1)	1 9 600	1 64 000
(2)	1 19 [illegible]	1 1[illegible]8 000
(3)	1 64 000	1 358 400
(4)	1 64 000	1 358 400
trypan red	no action	no action
Hg trypan red (1)	1 [illegible] 400	1 76 800
(2)	1 38 400	1 153 600
(3)	1 64 000	1 2[illegible]6 000
(4)	1 76 800	1 [illegible]8 400
acid fuchsin	no action	no action
Hg acid fuchsin (1)	1 14 400	1 51 200
(2)	1 3[illegible] 000	1 128 000
(3)	1 3[illegible] 000	1 179 [illegible]00

DISCUSSION

Dr A Gupta (Bengal) Asked whether there was any difference between the concentration of salvarsan and other compounds in the cerebro spinal fluid and the blood

Dr S L Sarkar (Bengal) Referring to the remarks in Dr Brahmachari s paper about the elimination of antimony in the administration of urea-stibamine and tartrate salts said that the curves referred to by Dr Brahmachari did not correspond with the curves dealing with elimination obtained by workers in the Chemical Examiner s Department He wanted more information regarding this point

He pointed out that the serum of the kala azar case was found to possess peculiar chemical properties such as in the formaldehyde reaction, rendered possible the decolorizing of methylene blue, and to a certain extent certain other dyes This chemical change in the serum should be studied As Dr Brahmachari s paper contained many valuable suggestions he suggested that he should try to have these worked out by young men under his supervision founding a school of antimony work in an institution founded by him

Dr L E Napier (Bengal) One of the main difficulties with which we are faced in research on the treatment of kala azar is due to the fact that antimony compounds *in vitro* have no effect on the parasite except in very strong solutions, furthermore as Noguchi has shown the addition of animal tissue does not increase this parasiticidal quantity of the antimony towards the parasite We are not able to use animals infected with *Leishmania* to test the efficacy of the antimony compounds as many of the compounds which are of great value in the treatment of human leishmaniasis have no effect on the leishmaniasis of animals The action of antimony is certainly an indirect one

I am not prepared to admit that stability of the compound and its therapeutic value are qualities which are necessarily inversely related My experience has however been that many of the stable compounds are of less therapeutic value than the less stable ones I still hope that chemists will at some future date produce a compound which is stable but is of higher therapeutic value than any of the compounds at present in use Stibosan is undoubtedly more stable than urea stibamine and it is of almost equal therapeutic value

Dr Brahmachari mentioned stibacetin as one of the compounds which he hoped might one day prove to be of value This is rather interesting as this compound the first of the pentavalent compounds to be used in the treatment of kala azar was prepared by Professor Schmitz of Dresden as long ago as 1915 and was used in the treatment of kala azar in Italy during the same year

Dr S Hata (Japan) replied We are carrying our research on the difference in the distribution in the blood stream and in the elimination from urine of salvarsan according to the difference of the reaction of its solution We have found however little differences With small animals as mice or rabbits it is nearly impracticable to see the difference in the distribution in the cerebral fluid of salvarsan after its intravenous injection Lately we are using salvarsan more directly introducing it into the spinal fluid for the treatment of the lesions of nervous system The direct injection into spinal fluid we can examine easily in the rabbits If any difference is found experimentally by our further researches I shall report it personally to Dr A Gupta

R B Dr U N Brahmachari (Bengal) replied I thank Dr Sarkar most heartily for his suggestion about the establishment of a school of research for carrying on investigations in chemotherapy with special reference to antimony and other therapeutic products The same suggestion has also been made to me by many other medical men Such an idea has been in my mind for sometime and I may state here in this connection that already such work is being done to some extent in my research laboratory I hope I shall be able to say one day that it has fully materialized and that a band of research workers are working in my laboratory under my guidance similar to what has been going on in the Bose Research Institute in Calcutta under Sir J C Bose or in the Institute of Science at Bangalore and then one of the ambitions of my life will be fulfilled

Regarding the excretion of antimony, I have observed that a large portion of it is excreted by the kidneys and a small portion only is excreted by the intestines

I think injections of an antimonial compound should always be given slowly to prevent any depression that may follow

The bio chemistry of the blood in kala azar has still to be worked out in greater detail I would only refer here to my work and those of others in India and China on globulins in kala azar serum

FURTHER EVIDENCE ON THE LIPOIDOPHILE ANTIGEN ANTIBODY REACTION

BY

KENJI TANIGUCHI, M.D.

Government Institute for Infectious Diseases, Imperial University, Tokyo

RECENT observations on the Forssman antigen have led to the conclusion that lipoids, especially phosphatides, can act serologically as antigen *in vitro* (the haptene of Landsteiner). Thus the Wassermann reagin and the Forssman antibody are called the lipoidophile antibody by the present lecturer Taniguchi in contrast with the proteinophile antibody, as the chemical character of antigens corresponding to the former antibody is lipoids, not proteins.

Further observations on this subject have developed a wide field in serology and immunology. Some interesting facts seem to have been added by workers in our laboratory at Tokio, to which I wish to refer here.

(1) It is known that sera from lepers frequently manifest a positive Wassermann reaction. According to the work of Murata and Tamiya of our laboratory, however, the so called Wassermann reagin which is contained in leprotic sera should be distinguished from the real syphilitic antibody for:

(*a*) According to the work of Tamiya, the lepra reagin does not fix complement with the Wassermann antigen in the ice box (0° to 5° C.), where the real Wassermann reagin or *syphilitic reagin* gives rise to a positive reaction. Out of 87 sera from leprosy patients examined, 45 manifested a positive Wassermann reaction by the ordinary incubation method. But when the ice box method is used for carrying out the Wassermann reaction (the mixture of the antigen, the cholesterinized heart extract, serum and complement is placed in the ice cold water for one and a half hours preceding the addition of the hæmolytic system), out of the 45 Wassermann positive leprosy sera 40 reacted negatively, the remaining five only manifesting a positive reaction by the ice box method as well as by the incubation method (Chart I).

Four out of these five were not simply leprotic, one patient being diagnosed as syphilitic and three others having anamnesis of gonorrhœa. The remaining one denied any anamnesis of venereal diseases.

Comparing the Wassermann reaction by the incubation method with that by the ice box method in 40 non leprosy patients, such striking discrepancies could not be observed as in the cases of leprotic sera, although a slightly smaller capacity of the sera to fix complement was observed in the ice box (Chart II).

CHART I

To show the difference in the Wassermann reactions in leprosy when the incubation method (black) and the ice box method (hatched) are used

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
37° W-R (B)	#	#	#	#	#	#	#	#	#	#	#	#	‡	‡	‡	‡	‡	‡	+	+	+	+	+	+
0° W-R (B)	−	#	−	−	−	−	−	−	−	−	#	+	−	−	−	−	‡	−	−	−	−	−	−	−

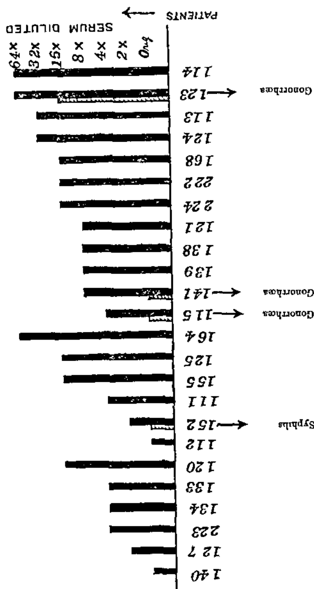
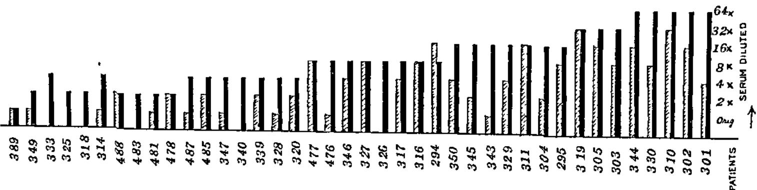


Chart II

To show the difference in the Wassermann reactions in syphilis when the incubation method (black) and the ice box method (hatched) are used.



(b) On the other hand the sera from leprosy patients frequently (39 out of 53 sera) fix complement when acted on with the saline emulsion of gynocardial oil (0 5 c c of rapid admixture of 1 0 c c of saturated alcoholic solution of *Oleum gynocardium* to 30 c cs of saline water to be used)

This is quite in contrast with the real syphilis reagin which usually gives negative results (Table I)

(c) Moreover, in the cases of leprosy sera divergence of result was frequently obtained between the Wassermann reaction and precipitation reaction of syphilis (Table I)

TABLE I

Difference between the various serological reactions of Lepra and Syphilis reagins

Reaction Patients	Wassermann R		Precipitation R		G O R.	Anamnesis of venereal dis
	37°C	0°C	Muratas mod of S g	M T P		
Yamamoto	+++	–	–	–	+++	–
Fujima	+++	–	–	–	+++	–
Inouye	+++	–	–	–	+++	–
(10) Nishikawa	++	–	–	–	+++	–
Kai	++	–	–	–	+++	–
Muramatsu	+	–	–	–	+++	–
Yamaji	+	–	–	–	++	–

TABLE I—*concld.*

Reaction Patients	Wassermann R 37°C	Wassermann R 0 C	Precipitation R Muratas mod of S g	Precipitation R M T R	G O R	Anamnesis of venereal dis
Yamada	++	—	—	—	+++	treated syphilis
Onose	+++	—	++±	positive	+++	—
Itagaki	+++	—	++	positive	+++	—
(15) Yoshida	+++	+++	+++	positive	+++	gonorrhœa
Otomo	++	+++	++	positive	+++	syphilis

It may, therefore, be concluded that the lepra reagin and the syphilis reagin, though equally manifesting a positive Wassermann reaction by the incubation method, should be serologically distinguished from each other.

(2) The results of Nimura's experiment showed that the autohæmolysin or Donath-Landsteiner's antibody of patients of paroxysmal hæmoglobinuria might present an example of lipoidophile antibody

It has been shown by Namba (D M W 1925, Nr 15, s 594) that the injection of organ emulsion of guinea pig, dog horse, and ox caused the rabbit to generate the autohæmolysin of Donath and Landsteiner.

After confirming the result of Namba, Nimura succeeded in producing the antibody by using lipoids from the organs of guinea pig and ox, digested with human serum (Table II)

METHOD

The preparation of the organ emulsion is the same as Namba's Ten c cs of the emulsion are injected into a rabbit intraperitoneally once a week (Table II)

Lipoid serum mixture —One part of minced wet organ is extracted with nine parts of 96 per cent alcohol, the whole is left standing for one week at room temperature and shaken several times

After this the extract is evaporated into a syrup, the original volume of human serum (diluted with normal saline 1 in 10) is added and left standing for one hour

TABLE II

Artificial production of autohemolysin in rabbits by injecting the mixture of organ lipoids and serum. (Three rabbits were used for each antigen, but only positive records are given here.)

Number of rabbits		1[illegible]					8			21			1				26		
Materials injected		Alcoholic Extract of											Saline Extract of						
		Guinea pig Kidney								Ox Heart			Guinea pig Kidney				Ox Heart		
Reactions examined		Control	Test in vitro: Test pro			Test in vivo	Test in vitro: Test pro.		Test in vivo	Test in vitro: Test pro		Test in vivo	Control	Test pro		Test in vivo	Control	Full serum	
		Full serum	Full serum	2x	4x		Control	Full serum		Control	Full serum			Full serum	2x			Test in vitro	Test in vivo
Before injection		−	−			−	−	−	−	−		−	−	−		−	−	−	±
Date of bleeding: Seven days after injection	I	−	±				−	−		−			−	+			−	±	
	II	−	+			++	−	−		−	±	++	−	±			−	±	
	III	−	+++	++	±		−	−		−			−	+			−	+	+
	IV	−					−	+	++				−	+	±	+			
	V																		
	VI																		

Twenty c cs of such lipoid serum mixture was injected into the peritoneal cavity of the rabbit once a week The rabbits had been examined for the accidental existence of autohæmolysin previous to the injection

Examination of serum (Test *in vitro*) One week after each injection rabbits were bled into test tube which was kept in the water bath at about 35° C As soon as the blood clotted, the serum was separated From a small portion of blood which was collected in citrated saline, the blood corpuscles were centrifugalized and washed three times with saline and a 30 per cent suspension was prepared

The mixture of 0 5 c c of serum and 0 1 c c of blood corpuscles suspension was placed in the ice cold water for 30 minutes, and after standing the tubes in the incubator at 37° C for two hours the results were recorded to be +++, ++, +, =, or –, according to the degree of hæmolysis

As a control, the same corpuscle mixture was left standing in the incubator for two and a half hours without cooling

Test in vitro —The root of the rabbit s ear was bound tightly with a rubber band, and the ear was dipped in the ice water for 15 minutes A few drops of blood were collected in a capillary test tube and centrifugalized As a control, an equal volume of blood was collected from the other ear previous *to cooling the fastened ear When the control serum showed no hæmolysis while the serum from the* cooled ear was tinted the results were recorded as positive

(3) Mizunuma discovered in human sera, a new lipoidophile antibody which is contained in the serum of blood type I (or O) and which gives rise to the positive complement fixation reaction along with the alcoholic extract of special human red corpuscles

This antibody is present in about 50 per cent (23 47) of sera of type I The antigen for this antibody is never contained in the blood corpuscles of the same type (Type I, or O) The blood type III (B) is also practically devoid of this antigen It is, however, contained in the corpuscles from types II and IV (Table III)

TABLE III.

Complement fixation of alcoholic extracts of blood corpuscles and Mizunuma's antibody (extracts from autolysed corpuscles)

Serum	Red corpuscles				Number of sera examined
	I O	II A	III B	IV AB	
I	0	22	2	23	47
II	0	0	0	0	29
III	0	0	0	0	26
IV O	0	0	0	0	7

This antibody is absorbed by the red corpuscles of types II and IV, but not by those of types I and III (Table IV)

TABLE IV

Absorption experiments of Mizunuma s antibody with various types of red corpuscles F=Forssman antigen (alcoholic extract of guinea pig heart) W—Wassermann antigen (alcoholic extract of ox heart cholesterol)

SERUM YOKOMATSU																					
		Before absorption				Serum was digested with blood corpuscles of type															
						I				II				III				IV			
Doses of complement		2	3	4	6	2	3	4	6	2	3	4	6	2	3	4	6	2	3	4	6
Antigens used	I	C				C				C				C				C			
	II	O	O	O	O	O	O	O	O	C				O	O	O	O	C			
	III	C				C				C				C				C			
	IV	O	O	O	O	O	O	O	O	C				O	O	O	O	C			

TABLE IV—*concld*

SERUM YOKOMATSU																					
		Before absorption				Serum was digested with blood corpuscles of type															
						I				II				III				IV			
Doses of complement		2	3	4	6	2	3	4	6	2	3	4	6	2	3	4	6	2	3	4	6
Antigens used—*concld*	F	C				C				C				C				C			
	W	C				C				C				C							
SERUM KOIZUMI																					
Antigens used	I	C				C				C				C				C			
	II	O	O	Tr	C	O	O	Tr	C	C				O	O	D	C	C			
	III	C				C				C				C				C			
	IV	O	O	Tr	C	O	O	Tr	C	C				O	O	Tr	C	C			
	F	C				C				C				C				C			
	W	AC	C			JC	C			AC	C			AC	C	C	C	C			
SERUM TAKAMATSU																					
Antigens used	I	C	-			C				C				C				C			
	II	D	JC			Tr	AC			C				Tr	AC	C	C	C			
	III	C				C				C				C				C			
	IV	D	AC			D	JC			C				Tr	Vm	C	C	C			

Thus this antibody seems to have some special relationship with the blood types II and IV, especially with the antigen A of the red corpuscles

But divergent results have been obtained in some exceptional cases of sera

This antibody is distinct from all known lipoidophile antibodies such as the Wassermann reagin or the Forssman antibody both quantitatively and qualitatively, as proved by absorption experiments

Methods

Washed sediment of blood corpuscles were left standing in the ice chest for about 48 hours for autodigestion One part of the autolysed corpuscles was extracted with ten parts of 96 per cent alcohol for ten days at room temperature Alcoholic extracts from fresh corpuscles are not convenient for the test, as they often lead to pseudo reaction (Table V)

Table V

Complement fixation of alcoholic extracts of blood corpuscles and Mizunuma's antibody (extract of fresh corpuscles)

Serum	Red corpuscles				Number of sera examined
	I	II	III	IV	
	O	A	B	AB	
I	6	96	58	126	2[illegible]3
II	1	1	1	2	183
III	1	6	2	4	124
IV O	1	0	0	1	42

To four parts of the extract one part of 1 per cent alcoholic solution of cholesterol was added Fresh admixture of 1 c c of this corpuscle extract and cholesterol mixture to 30 c cs. of normal saline

[illegible] as positive

Method for adsorption experiment —Equal volumes of serum and washed blood corpuscles were mixed and left in the incubator at 37° C for one hour and then the serum was separated for use

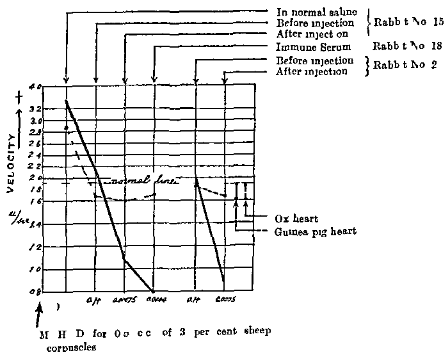
(4) The author is of opinion that the hypothesis of Ehrlich which separates antibodies into three groups (Rezeptor I, II III, Ordnung) on account of the different manner of reaction to corresponding antigens is not correct, but the

physico chemical processes which play the principal part in the antigen antibody reaction are always uniform because in the case of the lipoidophile antigen antibody reaction the single antibody manifests all three reactions i e neutralization precipitation and complement fixation with the corresponding antigen The necessity of electrolytes in the medium and the change of electrical charge of antigens is probably the principal factor which explains the mechanism of the proteinophile antigen antibody reaction

Shibata showed that in the case of lipoidophile antigen antibody reactions also such as the Forssman antigen antibody reaction or Wassermann reaction the emigration velocity of the antigen in the electrical field is diminished by the addition of the corresponding antiserum (Charts III and IV)

CHART IV

Influence of the Forssman antibody on Cataphoretic velocity of Forssman antigen



The potency of sera in this reaction accords quite well with their content of special antibody and this action of sera was prevented when the antibody was eliminated by digesting the serum with the corresponding antigen (Chart V) A definite period of time elapsed before this action of the serum was completed

(Chart VI) and this action took place at 37° C Thus the active moiety of the serum in the reaction must be the antibody contained in it

CHART V

Serum not treated
Treated c rabbits kidney
Treated c guinea pig kidney
Treated c sheep corpuscles Rabbit Serum N 1
Serum before injection

VELOCITY

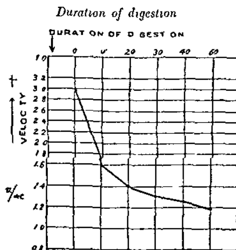
u/sec

normal line

M H D for sheep corpuscles

CHART VI

Duration of digestion



It should not escape notice that the normal serum itself alw ays reduces the electrical charge of suspensoids therefore the normal line should be drawn before making the test

The electrical charge of the emigration velocity of the antigen in the electrical field is examined by the method of electro cataphoresis devised by Seki

Antigen minced heart of ox (for Wassermann antigen) or of guinea pig (for Forssman antibody) is extracted in ten times its volumes of 96 per cent alcohol for three to four days at room temperature by shaking several times This extract is added slowly to 5 times its volume of normal saline To 1 c c of this mixture 0 05 c c of inactivated serum is added and left to stand at room temperature (about 18° C) for three hours *Now we are ready for the examination of electro* phoresis An object glass with a square chamber (400 deep) is filled with normal saline Two nibs of a calomel electrode are dipped one in each side of the chamber

One drop of the emulsion (mixture of antigen and serum) is then added to the *saline and the emigration velocity of the antigen particles is microscopically* examined (Zeiss C 4) by using an ocular micrometer

The electric current is 110 volts 0 003 to 0 004 ampere In order to differen tiate the migration of the particles it is convenient to add washed red corpuscles of the rabbit to the saline Here the red corpuscles of the rabbit are charged very weakly positive and move to the *kathode very slowly*

The H ion concentration of the saline water used should be just pH 7 0

For the Wassermann reaction Browning and Watson s method was used, and for the precipitation reaction of syphilis Murata's modification of Sachs Georgi reaction was used

Forssman antibody is prepared by injecting the water emulsion of guinea pig kidney into the rabbit and the antibody content of the sera was quantitatively examined by the hæmolysis of sheep corpuscles

The physico chemical process occurring in the lipoidophile antigen antibody reaction seems to be identical with those which manifested themselves in the proteinophile antigen antibody reaction

(Full reports will appear later in the Scientific Reports from the Government Institute for Infectious Diseases the Tokio Imperial University)

DEVELOPMENT AND DURATION OF IMMUNITY BY INOCULATION AND RE INOCULATION

BY

LIEUT COL W F HARVEY C I E M R C P E I M S (RETD)

AND

CAPTAIN K R K IYENGAR M D (Edin) D P H (Lond) I M S
Central Research Institute Kasauli

MUCH of the literature on the subject has centred around determinations of agglutinin increase or decrease Whatever may be the basis of relation between agglutinin content of the blood and protection their direct correspondence is disproved Our observations therefore of agglutinin production and protection simultaneously evaluated will be of interest in this connection

The organism which we have worked with as in most of our other work on immunity problems (1921 to 1923) is *B avisepticus* chosen because of its patho genicity to pigeons This organism has the additional advantage that as a member of the hæmorrhagic septicæmic group of organisms it is related to *B pestis* The pigeon has been chosen as our test animal because of its relative cheapness and therefore availability in numbers which would carry weight not only experimentally but also statistically We have preferred to rely on experiment based not so much on minimum lethal dose but on what we call a minimum lethal picture in which the minimum lethal dose and the multiples of the dose are all represented In every testing experiment done there was introduced a complete series of control animals which ensured that we had always at hand the equivalent of what statistically could be described as an alternate series for comparison The testing doses of the living virulent organisms used have reference to weight of the organism dry and have been determined by Brown s opacity tubes (1919) Two prophylactically treated pigeons were used for each testing dose and the doses were —0 000 000 005 0 000 000 01 0 000 000 05 0 000 000 1 and 0 000 000 5 mg We rely on actual protection from death as our indicator and for this purpose the animals were kept under observation for 72 hours The minimum lethal dose of the organism used in the test is between 0 000 000 000 1 and 0 000 000 001 mg We were dealing with so virulent an organism that—so at least we interpret the result—one single bacillus administered intravenously was sufficient to produce an acutely fatal septicæmia

In other words our experimental animals showed not the slightest resistance to the organism used

The last of our papers on immunity problems (1923) was of considerable import ance from the point of view of vaccino prophylaxis generally It dealt with the use of doses very much smaller than the original immunizing doses to restore im munity to original level In other words it brought forward the suggestion supported by experiment that re inoculation might be performed with minimal doses only and yet be effective The underlying idea was that although the animals which had been prophylactically immunized showed complete loss of immunity to test doses they were not identical with animals which had never been immunized at all they were sensitive to doses much smaller than non immunized animals and reacted to very small doses in the sense of developing a high degree of immunity With an extension of the idea of this sensitiveness to small doses we contemplated making a general statement—an animal once immunized is always immunized Such a statement would only be justifiable of course in a special sense and requires further proof Owing to the inability of the authors in 1923 to continue their experiments the further test of duration of the immunity brought about by the use of small doses in animals which had once been immunized but were no longer protected under the conditions of the test could not be carried out This oppor tunity has now come and we give the results of our trials intended not only to *demonstrate the point which was not tested before but to bring out and amplify* results already obtained

In this experiment 600 pigeons received inoculation and then served at stated intervals for test of protection Our endeavour was to use a sufficiency of animals *and a range of dosage which afford results that were securely significant* The experimentation was of a simple kind so as not to complicate the question by the introduction of too many factors at once Intravenous immunization and intravenous testing of the animals for protection were the methods employed because by these means one knows that the whole of the selected dose or doses have been administered The dosage to be used in immunization was based on our work on 'The relation between size of prophylactic dose and protection' (1923) The minimum dosage to give optimum protection in that investigation was 0 12 mg In our experimentation on '*single and fractional dosage* (1922) we showed two prophylactic doses afforded a better protection than a single dose and three doses better than two In this paper we have used the double prophylactic doses as the basis The first prophylactic dose of 0 5 c c contained the equivalent of 0 04 milligram and the second given 7 days later of 1 0 c c contained the equivalent of 0 08 milligram of dried bacterial substance

The agglutination response after the prophylactic inoculation was also investigated with a view to determining the degree of parallelism which exists between that development and protective immunity, and for this purpose two pigeons were tested in each series at each of the tests carried out

DEVELOPMENT AND DURATION OF PROTECTION

In our former experiments we used arbitrary first and second prophylactic doses of 0 35 and 0 7 mg In the present trials the results, while they generally confirm those previously obtained, show that a certain amount of protection is obtained as a result of the use of a single prophylactic dose of 0 04 mg (Table I), although not of the high degree which is evident after the administration of the second dose of 0 08 mg Three pigeons out of 10 survived as against 0 out of 10 for the control In our previous experiments we had not been able to show any protection after the administration of a single prophylactic dose This difference between the two trials may be due to our present use of an optimum minimum prophylactic dose as against the former arbitrary dose which was demonstrably larger than necessary A certain amount of temporary depression of the mechanism of immunity (negative phase condition) may have resulted from the use of too large a dose On the other hand the difference may only be accidental variation, such as is bound to occur in experiments of this nature Maximum protection was afforded within at least two weeks after the administration of the second prophylactic dose and by carrying out protection experiments every 30 days it was shown that this high degree of immunity lasted for 120 days, thereafter gradually decreased until there was almost complete loss of protection at the end of 180 days (Table II)

TABLE I

Showing in detail the degree of protection in pigeons seven days after the prophylactic administration intravenously of the first dose (0 04 mg) of B avisepticus *vaccine Two pigeons were used for each test dose, with the doses ranging from* 0 000,000 005 *mg to* 0 000,000,5 *mg Two pigeons were also used for each test dose as untreated controls, with the doses ranging from* 0 000,000,000,1 *mg to* 0 000 000,5 *mg The totals given in the table are only for the same range of doses for the treated and the untreated controls*

Dosage (mg) of the living organisms used in the test	SEVEN DAYS AFTER THE FIRST PROPHYLACTIC DOSE		CONTROLS UNTREATED	
	Survived	Died	Survived	Died
0 000 000 000 1			1	1
0 000 000 001			0	2
0·000 000 005	2	0	0	2
0 000 000 01	1	1	0	2
0·000 000 05	0	2	0	2
0·000 000 1	0	2	0	2
0 000,000 5	0	2	0	2
TOTALS	3	7	0	10

In other words our experimental animals showed not the slightest resistance to the organism used

The last of our papers on immunity problems (1923) was of considerable import ance from the point of view of vaccino prophylaxis generally It dealt with the use of doses very much smaller than the original immunizing doses to restore im munity to original level In other words it brought forward the suggestion supported by experiment that re inoculation might be performed with minimal doses only and yet be effective The underlying idea was that although the animals which had been prophylactically immunized showed complete loss of immunity to test doses they were not identical with animals which had never been immunized at all they were sensitive to doses much smaller than non immunized animals and reacted to very small doses in the sense of developing a high degree of immunity With an extension of the idea of this sensitiveness to small doses we contemplated making a general statement—*an animal once immunized is always immunized* Such a statement would only be justifiable of course in a special sense and requires further proof Owing to the inability of the authors in 1923 to continue their experiments the further test of duration of the immunity brought about by the use of small doses in animals which had once been immunized but were no longer protected under the conditions of the test could not be carried out This oppor tunity has now come and we give the results of our trials intended not only to demonstrate the point which was not tested before but to bring out and amplify results already obtained

In this experiment 600 pigeons received inoculation and then served at stated intervals for test of protection Our endeavour was to use a sufficiency of animals and a range of dosage which afford results that were securely significant The experimentation was of a simple kind so as not to complicate the question by the introduction of too many factors at once Intravenous immunization and intravenous testing of the animals for protection were the methods employed because by these means one knows that the whole of the selected dose or doses have been administered The dosage to be used in immunization was based on our work on The relation between size of prophylactic dose and protection' (1923) The minimum dosage to give optimum protection in that investigation was 0 12 mg In our experimentation on 'single and fractional dosage (1922) we showed two prophylactic doses afforded a better protection than a single dose and three doses better than two In this paper we have used the double prophylactic doses as the basis The first prophylactic dose of 0 5 c c contained the equivalent of 0 04 milligram and the second given 7 days later of 1 0 c c contained the equivalent of 0 08 milligram of dried bacterial substance

The agglutination response after the prophylactic inoculation was also investigated with a view to determining the degree of parallelism which exists between that development and protective immunity, and for this purpose two pigeons were tested in each series at each of the tests carried out.

Development and Duration of Protection

In our former experiments we used arbitrary first and second prophylactic doses of 0 35 and 0 7 mg In the present trials the results, while they generally confirm those previously obtained, show that a certain amount of protection is obtained as a result of the use of a single prophylactic dose of 0 04 mg (Table I), although not of the high degree which is evident after the administration of the second dose of 0 08 mg Three pigeons out of 10 survived as against 0 out of 10 for the control In our previous experiments we had not been able to show any protection after the administration of a single prophylactic dose This difference between the two trials may be due to our present use of an optimum minimum prophylactic dose as against the former arbitrary dose, which was demonstrably larger than necessary A certain amount of temporary depression of the mechanism of immunity (negative phase condition) may have resulted from the use of too large a dose On the other hand, the difference may only be accidental variation, such as is bound to occur in experiments of this nature Maximum protection was afforded within at least two weeks after the administration of the second prophylactic dose and by carrying out protection experiments every 30 days, it was shown that this high degree of immunity lasted for 120 days, thereafter gradually decreased until there was almost complete loss of protection at the end of 180 days (Table II)

Table I

Showing in detail the degree of protection in pigeons seven days after the prophylactic administration intravenously of the first dose (0 04 mg) of B avisepticus *vaccine Two pigeons were used for each test dose, with the doses ranging from* 0 000,000,005 *mg to* 0 000,000,5 *mg Two pigeons were also used for each test dose as untreated controls, with the doses ranging from* 0 000,000,000,1 *mg to* 0 000,000,5 *mg The totals given in the table are only for the same range of doses for the treated and the untreated controls*

Dosage (mg) of the living organisms used in the test	Seven Days after the First Prophylactic Dose		Controls Untreated	
	Survived	Died	Survived	Died
0 000 000,000,1			1	1
0 000 000,001			0	2
0 000,000 005	2	0	0	2
0·000 000,01	1	1	0	2
0 000,000 05	0	2	0	2
0·000,000 1	0	2	0	2
0·000 000,5	0	2	0	2
Totals	3	7	0	10

TABLE II

Showing in detail the duration of protection in pigeons as the result of prophylactic administration intravenously of two optimum minimum doses (0 04 *and* 0 08 *mg*) *at an interval of a week of* B avisepticus *vaccine Two pigeons were used for each test dose with the doses ranging from* 0 000,000,005 *mg to* 0 000,000,5 *mg Two pigeons were also used for each test dose as untreated controls, with the doses ranging from* 0 000,000 000,1 *mg to* 0 000 000 5 *mg*

Dosage (mg) of the living organisms used in the test	Days after the Second Prophylactic Dose																Controls Untreated	
	14		30		60		90		120		150		180		190			
	Survived	Died	Survived	Died	Survived	Died	Survived	Died	Survived	Died	Survived	Died	Survived	Died	Survived	Died	Survived	Died
0 000 000 000 1																		
0 000 000 001																		
0 000 000 005	2	0	2	0	2	0	2	0	2	0	2	0	1	1	0	2	0	16
0 000 000 01	2	0	2	0	2	0	2	0	2	0	2	0	0	2	0	2	0	16
0 000 000 05	2	0	1	1	1	1	2	0	2	0	1	1	0	2	0	2	0	16
0 000 000 1	1	1	2	0	1	1	1	1	1	1	0	2	0	2	0	2	0	16
0 000 000 5	1	1	1	1	1	1	1	1	0	2	0	2	0	2	0	2	0	16
Totals *	8	2	8	2	7	3	8	2	7	3	5	5	1	9	0	10	0	80

* The totals given throughout the table are only for the same range of doses for the treated and the untreated controls

The agglutination titre curve was not parallel to the
curve There is a marked diminution of agglutinins whil
tained at its maximum (Table III)

TABLE III

*Showing the highest dilution of serum of inoculated pigeons
scopic flocculation and its variation with time after d
tested in each experiment and four pigeons were used a*

Number of days to time of test	Highest dilution of serum giving agglutination		
7 days after the 1st dose	1 in 8	1 in 16	1 n
14 days after the 2nd dose	1 in 128	1 in 256	
30 " " " " "	1 in 64	1 in 64	
60 " " " " "	1 in 64	1 in 32	
90 " " " " "	1 in 32	1 in 16	
120 " " " " "	1 in 16	1 in 8	
150 " " " " "	1 in 16	1 in 8	
180 " " " " "	1 in 16	1 in 8	
195 " " " " "	1 in 16	1 in 8	

RESTORATION AND DURATION OF PROTECTION AFT

The usual method of re immunization is to repeat
dose In the present investigation we have sought to
under which immunity can be completely restored espe
re immunizing dose This is a most important matter, f
difficult it is to get large bodies of men voluntarily
inoculation and how very much more difficult it is to
previous experience by consenting to re-inoculation
series of doses for re immunization—the original optim
dose and doses which were 1-4 1 8, 1 16 and 1-32 of
of great satisfaction to find that these small doses, as
1 16th of the original gave maximum or nearly maxi

TABLE IV

Showing the duration of protection in pigeons after re immunization with two doses of B avisepticus *vaccine totalling* 0 12 *mg* (0 04 *and* 0 08 *mg*) *and with doses* 1 4*th*, 1 8*th*, 1 16*th and* 1 32*nd of this amount Two pigeons were used for each test dose, with the doses ranging from* 0 000,000,005 *mg to* 0 000,000,5 *mg Two pigeons were also used for each test dose as untreated controls, with the doses ranging from* 0 000,000,000,1 *mg to* 0 000,000,5 *mg*

Number of days to test after re immunization	Relative Amount of Vaccine used in Re immunization										Controls Untreated	
	Full dose		$\frac{1}{4}$th dose		$\frac{1}{8}$th dose		$\frac{1}{16}$th dose		$\frac{1}{32}$nd dose			
	Survived	Died	Survived	Died	Survived.	Died	Survived	Died	Survived	Died	Survived	Died
30 days after the 2nd dose .	7	3	8	2	8	2	6	4	2	8	0	10
60 „ „ „ „ „ .	8	2	8	2	8	2	5	5	1	9	0	10
90 „ „ . „ „ .	8	2	8	2	8	2	6	4	0	10	0	10
120 „ „ „ „ „	8	2	8	2	8	2	6	4	0	10	0	10
150 „ „ „ „ , ..	8	2	8	2	8	2	6	4	0	10	0	10
180 „ „ „ „ „ ..	4	6	4	6	4	6	3	7	0	10	0	10
210 „ „ „ „ „ ..	2	8	2	8	2	8	1	9	0	10	0	10

TABLE V

Showing the highest dilution of serum of re immunized pigeons which gave evident macroscopic flocculation and its variation with time after re immunization Two pigeons were tested in each experiment

Number of days to test after re immunization	Full dose.	¼th dose	⅛th dose.	$\frac{1}{16}$th dose	$\frac{1}{32}$nd dose
30 days after the 2nd dose	1 in 64 1 in 128	1 in 64 1 in 256	1 in 64 1 in 64	1 in 64 1 in 128	1 in 64 1 in 128
60	1 in 64 1 in 128	1 in 64 1 in 128	1 in 128 1 in 256	1 in 64 1 in 128	1 in 64 1 in 128
90	1 in 64 1 in 32	1 in 64 1 in 64	1 in 64 1 in 32	1 in 64 1 in 32	1 in 64 1 in 64
120	1 in 16 1 in 32	1 in 16 1 in 16	1 in 3 1 in 3	1 in 16 1 in 32	1 in 32 1 in 3
150	1 in 16 1 in 16	1 in 8 1 in 16	1 in 16 1 in 16	1 in 16 1 in 16	1 in 16 1 in 16
180	1 in 8 1 in 8	1 in 8 1 in 16	1 in 8 1 in 16	1 in 8 1 in 16	1 in 8 1 in 16
210	1 in 8 1 in 16	1 in 8 1 in 9	1 in 16 1 in 16	1 in 8 1 in 16	1 in 8 1 in 8

ensure the definite significance of our results By the use of a series of doses 1 4th 1 8th 1 16th and 1 32nd of the optimum in pigeons which had not been treated at all we were able to show that the immunity attained with these subliminal doses was not a maximum immunity The results for the above four doses were 30 days after administration of the second prophylactic dose, 4 survivors out of 10 3 out of 10 2 out of 10 and none out of 10

The agglutination results are shown in Table V The agglutinins are at a low level whereas protection is maintained at what is presumably its maximum

Conclusions

(1) Single doses of vaccine can produce immunity but not of so high a degree as two or three doses

(2) Some immunity can be obtained within a week of commencement of inoculation

(3) Full immunity is obtained within three weeks of the commencement of inoculation

(4) Re immunization may be carried out with doses which are smaller than even the smallest dose originally required to confer maximum immunity The immunity so conferred is as great and lasts as long as the original immunity

(5) There is no direct correspondence between agglutination titre and protection

(6) What applies to fowl cholera vaccine may apply to other vaccines as well

References

Brown, H C (1919) Further Observations on the Standardization on Bacterial Suspensions *Ind Jour Med Res* Vol VII No 1 p 238

Harvey, W F, Iyengar K R K and Christophers S R (1921) The Influence of Age and Temperature on Bacterial Vaccines First Part *Ibid* Vol VIII, No 4 p 715

Harvey, W F, and Iyengar K R K (1922) Virulence of a Micro organism and its Dependence on the Culture Medium *Ibid* Vol IX, No 4 p 726

Idem (1922) Virulence of the Organism as a Factor in the Efficacy of Prophylactic Vaccines *Ibid* p 730

Idem (1922) On the Immunizing Properties of Allied Organisms and Non specific Organisms *Ibid* p 736

Idem (1922) Retention of Virulence of a Micro organism kept in Sealed Culture *Ibid* Vol X No 1 p 190

Idem (1922) The Influence of Age and Temperature on Bacterial Vaccines Second Part *Ibid* p 19?

Idem (1922) The Advantages of Single and Fractional Dosage in Prophylactic Inoculation *Ibid* No ? p 424

Idem (1923) Increase of Dose as a Method of Making Use of Old Vaccine *Ibid* No 3 p 739

Idem (1923) The Development of Protection after Prophylactic Inoculation *Ibid* No 4 p 990

Idem (1923) The Duration of Protection after Prophylactic Inoculation *Ibid* p 1147

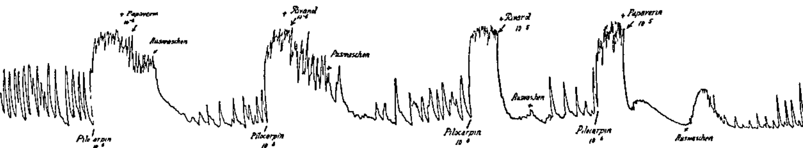
HARVEY, W. F., and IYENGAR, K. R. K. (1923) .. The Influence of Age and Temperature on Bacterial Vaccines Third Part *Ind Jour Med Res*, Vol XI, No 1, p 110

Idem (1923) .. On the Relation between Size of Prophylactic Dose and Protection *Ibid.*, p 113

Idem (1923) .. On immunization with relatively avirulent living organisms *Ibid* No 2, p 433

Idem (1923) Immunization by re inoculation, after a long interval, with a diminished dose of vaccine *Ibid*, p 437

CHART.



Colon, rat.
30. VIII. 27.
Curve No. 293.

Dr Vogel stated that if to a culture of *Entamœba histolytica* Yatren 105 in a concentration of 1 : 1 000 was added the amœbæ were after 15 hours still fit for further development. If Yatren was added for a longer period the amœbæ were destroyed.

The experiments of Dr Wagner showed the same results. He has also shown that Yatren 1 : 2 000 in Ringer solution did not kill *Entamœba muris* after five hours.

If to cultures of *Entamœba histolytica* Yatren 1 : 1 000 was added the amœbæ were still alive after 24 hours. If now to the media with the same amœba a fresh solution of Yatren was added the amœbæ were finally killed after another 24 hours time.

Rivanol in a concentration of 1 : 1 000 to 1 : 4 000 killed *Entamœba muris* in three to five hours. *Entamœba histolytica* was killed in 24 hours through a Rivanol solution of the same strength added to physiological saline (pH 7.4). That they were actually killed was proved by the fact that the same amœba in new culture media after 20 hours brooding did not recover. Rivanol 1 : 10 000 did not show any result in 48 hours time.

Kittens infected with *Entamœba histolytica* were treated with enemas of Rivanol 1 : 1 000 to 1 : 2 000 twice a day, each enema containing 10 c cs. The treatment was started after the disease reached the acme. Improvement was found already after 24 hours. The disappearance of the parasites was controlled by the microscope. The same results were obtained by administering the Rivanol per oral or combined per oral and rectal. The other Acridin derivatives were examined too, but they did not reach the activity of Rivanol.

There are experiments proceeding in treating human patients with per oral doses of Rivanol (keratinized pills) which are very successful although not finished as yet.

Finally pharmacological experiments were carried out to find an explanation of the quick disappearance of spasmodic contractions and tenesmus.

The isolated colon of the rat was poisoned with pilocarpin 10^{-6}. The simultaneous spastic contraction was released by papaverin 10^{-5}. Rivanol 10^{-5} served exactly the same way. Therefore we have to look upon Rivanol as one of our most powerful anti-spasmodics, which has the effective power of papaverin in releasing spastic contraction of the colon, but without disadvantages of an alkaloid of opium.

My conclusions are —

(1) 2 ethoxy 6 9 diaminoacridinlactate was clinically and experimentally proved as a specific compound against *Entamœba histolytica* and diseases caused by this parasite.

(2) This compound is a very powerful anti-spasmodic, useful in related symptoms of the intestines.

My thanks are due to Prof Schaumann and Dr Wagner for their kind permission to use their laboratory findings in this paper before their publication

REFERENCES

(1) PETER 1927) *Munch Med Woch*, 40
(2) WAGNER (1926) *Archiv f Sch u Trop Hyg*

SOME CLINICAL ASPECTS OF THE WASSERMANN TEST

EXPERIENCE IN CALCUTTA

BY

LIEUT COL R B LLOYD M A M B B Ch I M S,
Imperial Serologist Calcutta

THE object of this paper is to describe briefly certain clinical applications of the Wassermann reaction based on thirteen years' experience in the laboratory of the Imperial Serologist Calcutta of which the last seven years is the personal experience of the writer It has been found that with a reliable technique including adequate controls the Wassermann reaction is a not less valuable means of diagnosing syphilis in the tropics than in the West The Wassermann reaction not being a specific reaction in the bacteriological sense it is not surprising that positive reactions have been reported in persons suffering from other diseases from which syphilis has been believed absent as far as may be ascertained by clinical means The exclusion of syphilis by clinical observation is however a matter of extreme difficulty in some cases Further many of the reports of positive Wassermann reactions in non syphilitic conditions were based on imperfect technical methods, now discarded

The non syphilitic diseases which require consideration in this connection in Bengal are malaria yaws and leprosy Positive reactions have frequently been reported in the febrile stages of malaria Careful examination of the literature shows that excluding some of the results obtained by the older techniques the weight of evidence is against malaria causing a positive Wassermann reaction We have ourselves specially examined this question and find no evidence that malaria can cause a positive reaction Iyengar in Kasauli came to the same conclusion This is a matter of the greatest importance in clinical practice, as a tendency has been noticed on the part of physicians more particularly in Europe, to attribute to malaria an otherwise inexplicable positive Wassermann reaction in a person who has resided in the East This idea we believe to be incorrect, provided the result has been obtained in a reliable laboratory As is well known in a treated case of syphilis the obvious signs usually disappear before the positive Wassermann reaction which latter may only disappear permanently when intensive courses are given It follows that there are many persons, fewer no

doubt than in former days who exhibit no obvious signs of syphilis but whose Wassermann reaction is constantly positive These are generally though not invariably cases which have received insufficient treatment This type of patient may of course contract malaria The effect of anti malarial treatment is important here Reports as to the effect of quinine on Wassermann positive malaria cases are not entirely concordant but the general experience is that it has no effect on the serum reaction whereas adequate anti syphilitic treatment will often remove the positive reaction in such cases The inference that the positive Wassermann reaction is due to syphilis and not to malaria is we think clear The idea that malaria might cause a positive Wassermann reaction appears largely to have originated from the address given to the British Medical Association in 1907 by the late Professor Wassermann in which he stated that his assistant Dr Meier in association with Bonfiglio in Italy, had found this to be the case These results of Meier and Bonfiglio were never published The bulk of modern observers are against this idea There are however a few reports from reliable workers using modern techniques of Wassermann observations on persons who were subject to relapsing malaria and who constantly exhibited positive Wassermann reactions during the paroxysms the reaction returning to negative during the afebrile periods This result might seem to suggest that malaria can cause a positive Wassermann reaction On the other hand it might be due to the provocative action of the fever upon quiescent syphilitic cases which we know may have a negative reaction in the later stages even though syphilitic lesions may actually be present The usual experience that the positive Wassermann reaction outlasts the clinical signs is thus not without exceptions This raises the question of the effect of induced malaria given in the treatment of general paresis more particularly the effect upon the serology of such cases The writer has no experience of this as in Bengal a case of general paresis is hardly ever met with

Whatever may be the effect of malaria on syphilis the practical point for the serologist is to ascertain if malaria gives a positive reaction in the absence of syphilis by the actual technique that he uses This is in the first one of the most important of the controls in the test We believe on the basis of a long experience devoted specially to this question that the Wassermann method used in our laboratory may be guaranteed to yield a negative with a non syphilitic malaria case no matter what the type or stage of the malaria may be In any case there would seldom be any necessity to examine by the Wassermann reaction the blood of a malarial case *during the febrile period*

Yaws as is well known is caused by a spirochaete similar to though believed to be distinct from the causative agent of syphilis A positive Wassermann reaction is met with as a regular phenomenon in generalized yaws This is a matter of no great importance as yaws is comparatively uncommon in Bengal and when met with its recognition offers little difficulty The yaw is a hypertrophic type of lesion not met with in syphilis These consist histologically of epidermal abscesses

swarming with spirochætes The late ulcerative lesions of yaws, which may be similar to syphilitic lesions are very uncommon

The case of leprosy is more complicated Many modern Wassermann techniques including our own which quite easily exclude false positives due to malaria will constantly yield positive reactions in leprosy The question arises whether these are due to leprosy or to co existing syphilis In association with my colleague Dr Muir I have made an extended enquiry into this question

Broadly speaking the earlier observers found a high percentage of positive fixations in leprosy much higher than that met with in any other non syphilitic disease (except yaws) the percentage also being much higher than associated syphilis would appear likely to be able to account for Most observers also found many more positive fixations in the dermal types of leprosy than in the neural types The general impression was then that a positive Wassermann reaction is an integral part of many cases of leprosy and is not due to associated syphilis Our own work taken in conjunction with the recent results of Kolmer in America has led us to quite different conclusions We have specially studied the effect of anti syphilitic treatment on Wassermann positive leprosy cases Our research included Wassermann tests on over a thousand cases of leprosy An accurate diagnosis is of course the first essential This was made by my colleague Dr Muir who found leprosy bacilli in every one of the dermal types and anæsthesia or other definite evidence of nerve involvement in the neural types We find as will appear that anti syphilitic treatment has a very marked power of removing the positive Wassermann reaction in leprosy The reason we think that earlier workers found it had little influence is that for the most part they were treating very old standing cases of leprosy with anti syphilitic treatments which would not now be considered adequate for a case of syphilis We on the other hand have treated earlier or milder cases of leprosy with intensive anti syphilitic courses We regard the exclusion of syphilis by the history and by clinical observation as a process so uncertain as applied to illiterate patients that it is inadvisable to use it for research purposes As a basis for our conclusions we have preferred to rely on the syphilis rate of the population Not very much has been done as yet in India in the way of Wassermann surveys but it is well known that the syphilis rate of the Calcutta hospital population is in the neighbourhood of 15 to 20 per cent Consequently if we find this percentage of positive Wassermann reactions in a non syphilitic disease we need look no further for its cause

We will very briefly state some of our most recent results —

(i) Of 341 early cases of skin leprosy 54 showed a positive Wassermann reaction (15 8 per cent)

(ii) Of 87 early cases of nerve leprosy 12 showed a positive Wassermann reaction (13 8 per cent)

(iii) Of these two groups 21 Wassermann positive cases were given anti syphilitic treatment and in every one of these cases the reaction became negative (100 per cent)

The facts which emerge from these figures relating to early cases are firstly that there is no difference in the Wassermann positive rate between the dermal and the neural types, secondly that in both types the Wassermann positive rate is within the syphilis rate and thirdly that in every single one of these 21 cases anti syphilitic treatment promptly removed the positive Wassermann reaction All these Wassermann positives were then clearly caused by associated syphilis

In the later stages of skin leprosy we found that 50 per cent or more of the cases showed positive Wassermann reactions Of these three fourths yield to anti syphilitic treatment Comparatively recently Kolmer in America has perfected a new Wassermann technique one of the leading features of which is the wide gap between the antigenic and anti complementary doses of the antigen employed Briefly Kolmer finds that with his new method the Wassermann reaction is never positive in leprosy unless syphilis be present in addition Where Wassermann techniques are employed in which there is not so wide a gap between the antigenic and anti complementary doses of the antigen some cases will react positively which would yield negative results with the 'wide gap' method These are false positives due to experimental factors In addition to these there are at any rate in India a large number of positive Wassermann reactions in leprosy which are due to associated syphilis These two factors make up the 50 per cent or more of positive Wassermann reactions met with in the advanced skin types of leprosy We have not as yet fully worked out the problem but we find that as stated three fourths of all such positives yield to anti syphilitic treatment and may therefore be attributed to syphilis How can we explain this high proportion of associated syphilis—two to three times the syphilis rate—thus met with in a disease which in its milder phases shows no evidence of special association with syphilis? We think the most probable explanation is that tissues damaged by the syphilitic virus form an excellent soil for the advance of the leprosy process and that those mild cases which have syphilis associated soon cease to be mild and tend rapidly to pass into the graver forms of leprosy thus producing an accumulation of the Wassermann positive cases in the graver types If the question then be asked Is a positive Wassermann reaction met with in leprosy? the answer is No if the 'wide gap' technique be used and *if the patient is free of syphilis*

Dr Muir and I have ascertained that serological examination for associated syphilis and its removal when present is indispensable in the proper management of a leprosy clinic It is obviously undesirable to subject to anti syphilitic treatment a leper who has no syphilis and whose positive Wassermann reaction is due to experimental factors It is therefore essential if the Wassermann test be used for this purpose to employ the 'wide gap' technique Facilities for the performance of the Wassermann test by reliable workers are in India at present limited to a comparatively few large centres The complicated nature of the test renders it quite unsuitable for use in out-station laboratories with few facilities It is absolutely necessary as the Medical Research Council in London strongly urged that Wassermann work be centralized in the hands of *experienced laboratory workers* The

central institutes of India, perhaps a dozen in number, are able to cover a surprisingly large area, but if it be decided to establish a large number of small leprosy clinics in rural areas, it will probably be found preferable, on the score of economy and efficiency, to employ a well adjusted flocculation test, such as that of Kahn for the detection of associated syphilis in a leper We have now abundant clinical evidence that the removal of syphilis is the first requisite in the treatment of leprosy and Dr Muir now effects this by using Hg_{33} a new mercury preparation now known as Avenyl This has the great advantage that it produces no serious reactions which may be dangerous in leprosy, and further it is soluble in hydnocarpus oil and esters The effect of this combined treatment is very remarkable, the clinical improvement in some cases being quite extraordinary Dr Muir is so impressed with the role of syphilis in delaying cure in leprosy that he never uses a Wassermann positive case as a gauge for the activity of any new drug to be tested

In the Calcutta hospitals the Wassermann reaction is now very greatly used in the diagnosis of medical cases and every patient admitted to hospital under the care of the Director of the Calcutta Tropical School is subjected to this test as a routine As might be expected many cases of syphilis have been brought to light of which there was not the faintest suspicion clinically the correctness of the Wassermann finding being shown by the striking improvement which occurred on anti syphilitic treatment Syphilis is very common indeed in clinical practice in Bengal The clinicians present will be able to speak in detail on this As a serologist I will just quote a few cases

The following are some cases in which the discovery of a positive Wassermann reaction altered the whole outlook —

Case 1 Cerebro spinal meningitis—A man was admitted to hospital with cerebro-spinal fever the diagnosis being confirmed by the discovery of the meningococcus With anti meningococcal serum some improvement occurred but later the patient became comatose and was evidently dying A Wassermann test was taken and the reaction was found positive Anti syphilitic treatment altered the whole appearance of the case and the man recovered

Case 2 Urinary type—A middle aged man was admitted for hæmaturia The only abnormality detected was moderate leucopenia Cystoscopic examination was negative The Wassermann reaction was found to be strongly positive On anti syphilitic treatment the hæmaturia ceased entirely

Case 3 Dysenteric type—A young Hindu male was suffering from dysenteric symptoms of the bacillary type The usual treatment failed to relieve him His epitrochlear glands were enlarged and he showed old scars His Wassermann reaction was taken and found to be strongly positive With anti syphilitic treatment the dysenteric symptoms entirely disappeared

Case 4 Pulmonary type—A middle aged Hindu male was suffering from fever and cough The spleen was slightly enlarged and the right lung showed signs of early tuberculosis Malarial crescents were found in the blood and under quinine some improvement occurred He still remained very weak and anæmic Examination for hookworm was negative His Wassermann reaction was found to be strongly positive, and on a combined quinine and anti syphilitic treatment he completely recovered with the disappearance of all lung signs

Case 5 Anæmia type—These are very common A young Hindu male was admitted for anæmia debility and tingling of extremities. His blood showed r b c, 2½ millions, white cells, 5,000,

hæmoglobin, 40 per cent No other signs and no history of venereal disease His Wassermann reaction was strongly positive On anti syphilitic treatment he recovered completely

Case 6 Cardio vascular type—These are very common A man, aged 42, was admitted with a double aortic lesion secondary mitral regurgitation and heart failure A fortnight's digitalis partially re established compensation but he was unable to lie down in bed No history or signs of venereal disease could be obtained His Wassermann reaction was strongly positive He was then put on a combined treatment of digitalis and mercury by the mouth The signs of heart failure disappeared in one month He was unable to tolerate intravenous arsenic, and only made a partial recovery

Case 7 Nerve type—A Hindu male was admitted for epileptiform fits His age and the short history suggested a Wassermann test which was strongly positive After anti syphilitic treatment the man has remained free from fits for two years

For the above case notes I am greatly indebted to Lieut Col J W D Megaw C I E I M S, and Major J C De, I M S.

As evidence of the value of routine Wassermann tests these cases speak for themselves

As to the part played by syphilis in delaying or preventing the cure of other diseases, the case of leprosy has already been instanced There are many other examples of this e g there is little doubt that Wassermann positive kala azar cases fail to respond to the specific antimony treatment as freely as usual, and operations for glaucoma tend to fail in a Wassermann positive patient

I am also indebted to Major H Hingston I M S, for some extremely valuable notes on various aspects of syphilis in the Calcutta hospitals Space will only allow me to include one of his notes He informs me he has notes of 18 consecutive cases of ascites associated with marked anæmia and a large hard spleen In these eighteen cases, the Wassermann reactions were as follows — positive, 7, negative 5, doubtful, 6 In addition to the large number of positives, the percentage of doubtful reactions was high 33 per cent This is much higher than the usual proportion of results classed as doubtful, and very possibly some of these would have been reported positive had provocative injections been employed Major Hingston ascertained that the post mortem appearances were macroscopically those of ordinary cirrhosis of the liver, without special evidence of syphilis

He found that in this type of case the patients react badly to treatment being extremely intolerant of arsenic injections The exact connection between syphilis and this type of case is a matter of great importance This we hope to work out more fully later

The Wassermann reaction marvellously accurate as it is, is not absolutely infallible It has attained its immense reputation by its agreement with clinical findings in an overwhelming proportion of cases, and by that standard, which is the only standard possible it must always be judged It follows that the ultimate diagnosis of a case must rest with the clinician, who is always ready to appreciate, amid the complexities of clinical diagnosis the value of a test which, when properly performed gives almost always a correct indication Given skilled workers, variations between different Wassermann results obtained by different techniques

are due to differences in quantitative adjustment, i e , a Wassermann system which is highly sensitive is probably not entirely free from false positives Conversely, a system of deficient sensitiveness will avoid false positives, but will, on the other hand miss some cases of undoubted syphilis (false negatives) Between these two difficulties the serologist must steer his course according to his experience

All serologists agree that the use of over delicate Wassermann systems is inadvisable, if a somewhat sensitive system be used, all partially positive reactions should be viewed critically if the case is one for diagnosis It is here that experience in the interpretation of the experimental result is of so much importance

LES MÉTHODES DE VERNES EN GÉNÉRAL ET LA SYPHILIMÉTRIE EN PARTICULIER

PAR

MAJOR V G F LABERNADIE,

Chef du laboratoire de Pondichéry, Establissements français dans l'Inde

La méthode de Bordet Wassermann et ses dérivées utilise un systeme hemolytique dont les variations colorimétriques servent d'échelle pour l'expression des résultats Tout le monde est d'accord pour constater que cette échelle ne peut se diviser qu'en une dizaine de degrés allant du positif fort au négatif complet

Au moment des découvertes de Bordet et Wassermann et des recherches d'Ehrlich la theorie en vigueur etait une sorte de chimie du sérum ou les antigenes ambocepteurs alexines s'unissaient suivant certaines lois

Les recherches de ces vingt dernieres années se sont orientées vers les états colloidaux et leur floculation et l'opinion actuelle est que les réactions dites autrefois de fixation du complement sont surtout d'ordre physico chimique et d'essence colloidale

La méthode de Vernes est basée sur l'observation directe des troubles colloidaux des sérums et liquides cephalo rachidiens pathologiques non plus par le médium *d'un systeme hemolytique a dix degrés mais grace à un photometre de grande* precision et de parfaite sensibilité puisque sa gamme comporte 150 divisions

D'autre part les conditions colloidales ont éte etudiées avec le plus grand soin les suspensions l'ajustement des divers réactifs ont été l'objet d'une mise au point précise qui permet de rendre rigoureusement comparables les séries de reactions du meme ordre physique Rappelons au contraire combien la réaction de Bordet Wassermann est inconstante et souvent paradoxale car elle est difficile a regler dans ses divers élements biologiques

* * * * * * *

Essentiellement voici le principe de la methode de Vernes Le réactif choisi est de l'extrait de cocur de cheval epuisé successivement par le perchlorure d'éthylene puis par l'alcool d'ou son nom de *perethynol* Fabriqué dans les laboratoires de l'Institut Prophylactique il est toujours identique a lui meme—On l'utilise apres l'avoir melange par des manipulations speciales à de l'eau bi distillée en proportion de 1 pour 5 5—Telle est la suspension granulifere a grains homogenes dans laquelle les serums decantés centrifugés chauffes 30 minutes à 55° vont

provoquer des troubles quelquefois visibles à l'oeil nu et toujours mesurables au photometre

L'ajustement des réactifs est en effet tel que le mélange perethynol+sérum garde la meme densite optique si le sérum est sain ou au contraire flocule et s oppose au passage de la lumiere si le sérum est syphilitique et seulement s il s agit de syphilis

La technique de la reaction est simple dans un tube à hemolyse à 0cc8 de sérum chauffe on ajoute 0cc4 de suspension de pérethynol Dans un autre tube le même serum (0cc8) et de l eau alcoolisée (0cc4) constituent le témoin car la dilution de l alcool a éte choisie de telle sorte qu elle ait la meme densité optique que le suspension de péréthynol et quelle ne provoque aucune floculation dans les sérums

Apres quatre heures de repos a la temperature de 25 a 30° on fait la lecture au photomètre du tube à reaction et du témoin La différence entre les deux lectures donne la densite optique de la surfloculance due à la syphilis Ce chiffre varie entre 0 and 150 et meme au delà Il donne de precieux renseignements pour le diagnostic pour le dépistage des cas anciens insuffisamment traités enfin et surtout pour le controle du traitement institué

Une analyse mensuelle est nécessaire pour bien suivre le malade en traitement Les chiffres reportes sur un graphique forment une courbe qui donne des indications importantes pour intensifier les doses continuer ou changer de médicament etc Cette série d analyse permet aussi d etre certain de la guérison du malade Si après un traitement bien controle et terminé par des injections arsenicales le malade montre un Vernes négatif dans son serum pendant huit mois consécutifs et si à cette date son liquide cephalo rachidien est normal l expérience menée depuis 15 ans sur 70 000 cas montre qu on doit considerer le malade comme guéri

Telle est la méthode de Vernes d un puissant intérêt pour le diagnostic de la syphilis dans le sérum Mais on sait qu une syphilis plus ou moins ancienne peut se localiser sur les centres nerveux laissant encore ou non des traces dans le sérum une legere modification a la technique exposée ci dessus permet de rechercher la floculation dans le liquide céphalo rachidien avec les memes appareils

Enfin une vieille syphilis nerveuse peut ne donner plus comme symptomes que de l Hyperalbumino rachie Le photomètre de Vernes permet encore de mesurer le taux de cette albumine

La meme installation de base permet donc de rechercher tous les symptomes humoraux de la syphilis

* * * * * * *

Mais A Vernes ne s en est pas tenu là et après de longues études avec ses collaborateurs Bricq et Gager il a mis au point le diagnostic sérologique de la tuberculose à l aide d une suspension colloidale de resorcine toujours avec les memes appareils Cette floculation a montré en outre un fait curieux Comme le Bordet Wassermann la réaction de Vernes dans le serum sanguin est négative

dans les premiers jours du chancre syphilitique Et, justement, la réaction à la résorcine mise en œuvre à la suite de l'apparition d'un chancre syphilitique est alors toujours positive mais passagère, disparaissant à peu pres au moment où la Vernes péréthynol devient positif (à moins évidemment que le sujet soit tuberculeux, auquel cas le Vernes résorcine n'a pas de signification quant à la syphilis primaire)

* * * * * * *

Tels sont les immenses services que rendent les méthodes de Vernes pour l'application des quelles la République Française, le Département de la Seine et la ville de Paris ont fait bâtir un immense immeuble appelé Institut Prophylactique et dont le but est de poursuivre l'extinction de la syphilis Au Comité de Direction figurent les plus grands noms politiques, juridiques, scientifiques, littéraires, industriels, financiers, civils et militaires, religieux et laïques—De fortes subventions sont allouées et sont nécessaires car l'Institut Prophylactique examine et traite gratuitement En dix ans 70 000 malades ont reçu un million de consultations et 700 000 déterminations sérologiques ont été pratiquées soit à l'Institut Prophylactique de la rue d'Assas soit dans les 20 dispensaires annexes de Paris et de sa banlieue—sans compter 40 grandes villes de France qui ont des organisations analogues

Les Colonies Françaises et plus de 25 pays étrangers ont suivi cet exemple *créant une soixantaine de dispensaires—laboratoires C'est qu'en effet la méthode* de Vernes est applicable partout et particulierement dans les pays chauds M Leger (1) a montré qu'avec des modifications de détail dans l'appareillage et l'emploi d'eau glacée on peut opérer à la température de 25° que les expériences de Vernes et ses collaborateurs ont montré être la plus favorable

A Pondichéry, nous utilisons depuis quelques mois SANS REFRIGERATION la méthode de Vernes à la température ambiante qui est d'environ 30° et elle nous a donné toutes satisfactions

* * * * * * *

Il est à souhaiter que ces précieuses méthodes de diagnostic et de *contrôle* se généralisent et que partout on travaille dans le même sens et avec les mêmes succès que l'Institut Prophylactique de Paris dont, pour citer un témoignage certes impartial, Wassermann lui même, le père de la serologie, disait déjà en 1922 " Il n'est pas un établissement au monde, qui puisse, même de très loin, offrir une documentation comparable à celle ci, et sous le rapport du nombre des malades et sous le rapport de leur contrôle à longue echéance "(2)

INDEX BIBLIOGRAPHIQUE

(1) LEGER, M (1925) La Syphilis dans les Colonies Françaises *Rev Méd Hyg Tropicales,* No 3

(2) VERNES, A (1927) Rapport du Directeur à l'Assemblée Genérale du Comité de Direction, de l'Institut Prophylactic, Juin

LA RÉACTION DE BORDET WASSERMANN SANS ÉTUVE

PAR

MAJOR V. G. F. LABERNADIE

Chef du laboratoire de Pondichéry, Établissements français dans l'Inde

Bien que la réaction de Bordet Wassermann ne soit pas exempte de tout reproche, elle est pratiquement d'une réelle importance dans le diagnostic de la syphilis, aussi est elle universellement employée.

La réaction originale étant un peu compliquée, des procédés dérivés ont été mis au point et le Hecht Bauer par exemple est d'une grande simplicité et d'un facile emploi. Cependant il n'est guère sorti des laboratoires et peu de praticiens l'effectuent dans leur cabinet. Cela se comprend pour l'Europe et les grandes villes coloniales munies de nombreux instituts. Mais lorsqu'il s'agit de vastes provinces où la plupart des Médecins pratiquent loin des centres, il est regrettable qu'ils soient obligés de se priver de l'appoint du laboratoire dans la discussion d'un cas douteux.

Pour appliquer le Hecht Bauer ils auraient cependant facilement le petit matériel nécessaire; dans la plupart des Colonies et particulièrement dans l'Inde on peut se procurer des moutons et ponctionner leur jugulaire, mais en général il manque toujours aux petits postes, en raison de son prix élevé, une étuve à température constante pour maintenir les tubes à réaction à 37 C.

En vue de la diffusion du Bordet Wassermann (Hecht Bauer) particulièrement aux colonies, nous nous sommes demandés si la température de 37 C était rigoureusement indispensable et si l'on pourrait utiliser la chaleur atmosphérique ambiante.

En Guyane Française (Amérique du Sud) nous avons opéré sur 228 sérums leur appliquant le procédé de Hecht Bauer à l'étuve à 37°C et à la même heure en laissant les tubes sur la table du laboratoire à la température ambiante qui était de 28 à 30°C. Dans *ces conditions nous avons obtenu entre les deux séries 206* concordances soit 90 pour cent(1).

Nous avons repris ces essais à Pondichéry où la température ambiante était de 32 à 35°C et pendant plusieurs semaines nous avons eu 100 pour cent de concordance.

On peut donc conclure que si au dessous de 30°C il y a quelques discordances, au dessus de 32°C au contraire la réaction est aussi valable qu'à l'étuve à 37°C.

Bien évidemment cela ne condamne pas l'étuve mais il nous semble que ces résultats doivent permettre à un praticien isolé dans les régions chaudes de l'Inde, en présence d'un cas suspect de syphilis, d'effectuer, sans etuve, un *Hecht Bauer dont le résultat prudemment interprété* pourra faire pencher la balance du diagnostic

INDEX BIBLIOGRAPHIQUE

(1) LABERNADIE V G F (1926) Diagnostic sérologique de la syphilis aux colonies *Rev Méd Hyg Trop*, No 6

DISCUSSION

Major H Stott I M S (United Provinces) We can all greatly appreciate the high authority with which Major Lloyd speaks on the Wassermann reaction I would like to ask a few questions and to make one or two comments Firstly, what does he mean by a proportion of positive Wassermann reaction in a hospital population of 15 to *20 per cent ? Does he mean this percentage of positive sera amongst the sera sent to him* for Wassermann reaction being suspected of syphilis by the staff or does he mean that a special survey of every patient attending hospital or in hospital was made ? Can he *give us any information regarding the percentage of sera at first negative which have* become positive after a provocative injection of salvarsan ? Are any figures available as to the percentage of cases which have a positive cerebro spinal fluid with a *negative* blood serum ?

Now that malaria and leprosy have been excluded so satisfactorily from the positive Wassermann reaction group I would ask what two diseases are under suspect as a cause of a positive Wassermann reaction ? There is relapsing fever in which, I believe the reaction during the febrile phase approaches 85 per cent

I feel it is important that a definite expression of opinion should be laid down by an authoritative body that Wassermann reaction should only be carried out in central laboratories where skilled workers and the necessary equipment and material is freely available as is advised by the Medical Research Council

I entirely agree with Major Lloyd's observation that the removal of a syphilitic infection whether obvious or latent is essential for the full efficient treatment in clinical cases With Dr Muir his observations have been mainly concentrated in leprosy and with Colonel Knowles in kala azar My own experience has been similar in a disease of a somewhat different nature, namely, *diabetes In fact, it is indeed becoming obvious* that in any case when the progress of the disease under treatment is not satisfactory or, indeed is a routine in any serious condition, a Wassermann reaction should be undertaken

Professor T Taniguchi (Japan) I quite agree with Major Lloyd that great care must be taken in carrying out the Wassermann reaction I should like to advise that [illegible] for by this method the [illegible] would be eliminated

Professor S Hata (Japan) [illegible] cases of rat bite fever we found very strong hæmolysin, besides the Wassermann reaction. In experimental rat bite fever in the rabbit we can produce very easily such a chronic disease, in which

a positive Wassermann reaction and hæmolysin (up to 3-4000 X dilution) is to be observed To study the relation between these two reactions of the serum, we treated those animals having a chronic course of the disease with salvarsan and found that the Wassermann reaction disappeared first, while the hæmolytic power of serum remained and resisted the treatment much longer So long as the hæmolysin remains, the infection is not yet completely cured, as the recidive of the Wassermann reaction occurred in such cases Both these reactions are surely different in their nature, but what relation they have to each other has yet to be studied

Capt K R K Iyengar, I M S (B India) Regarding the point raised by Major Lloyd as to the occurrence of a positive Wassermann reaction in relapsing fever, I would like to point out that 90 per cent of relapsing fever cases give a strong positive reaction +++ during the febrile period and this strong positive reaction becomes negative during the convalescent stage Some cases relapsed and these, again gave a strong positive reaction which became negative when the temperature came down to normal

Major R B Lloyd, I M S (Bengal) In reply referred to Dr Vernes' splendid work in Paris and said that he thought that a great deal of his success was due to the fact that he had realized many years ago the absolute necessity for intensive courses in the treatment of syphilis He asked Major Labernadie to explain how 150 different grades of opacity could be demonstrated by the Vernes' test

In reply to Major Stott, Major Lloyd stated that the syphilis rate of approximately 15 to 20 per cent of the Calcutta hospital population was an estimated figure arrived at from the records of his department as a result of various Wassermann enquiries into non syphilitic conditions, also from the general medical wards of the Calcutta hospitals It did not refer to the Wassermann positive percentage found in cases sent with a suspicion of syphilis No accurate Wassermann surveys had yet been done in Bengal With regard to Wassermann tests after provocative injection in a relatively small proportion of cases, a second Wassermann test (after a provocative injection) is asked for, more particularly of course where the clinician has a strong suspicion that the case is syphilitic

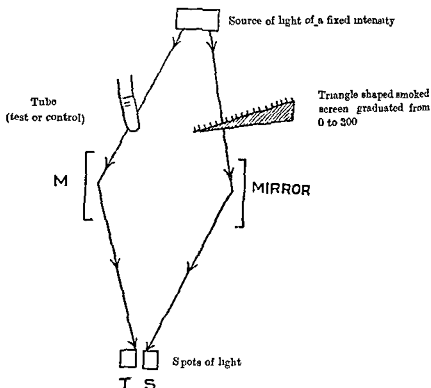
Not very many specimens of cerebro spinal fluid are now received for Wassermann test Patients very frequently object to the operation Major Lloyd referred to recent experience at the London Venereal Clinics which tend to use spinal puncture less frequently than formerly While a perfectly sound proceeding, it is found in practice to increase the 'defaulter rate' of these clinics, the slight advantage gained being greatly outweighed by the fact that other patients are afraid of the operation, and desert the clinic before their treatment is completed.

Major Lloyd thanked Dr Taniguchi for his information that the ice-box technique is very valuable when performing Wassermann tests in leprosy, and in referring to Professor Hata's interesting communication on the Wassermann reaction in rat-bite fever said that up to the present he had only been able to test three cases, the reaction being positive in two and negative in one

Major V. G F. Labernadie (French India) The principle of the V B Y Photometer which explains its sensibility, lies in the presence of a large scale to measure the

least difference which occurs between the optical densities of the test tube and the control tube

The light at S is made variable by means of the screen which permits the intensities to be equalized and thus allows of a measurement with T by means of a graduation of the screen



For example, we take a reading on the test tube and after equalization we see 250 on the screen *On the control tube, we find 160 , 250—160=90, which is the syphili metric index* This means the measurement of the intensity of the flocculation which has occurred, which is proportional to the infection

If the same number is found in the two readings, the difference (zero) means no flocculation and thus no infection

RABIES AND ANTI-RABIC TREATMENT.

THE ACTION OF ETHER ON THE RABIES VIRUS

BY

LIEUT COL J CUNNINGHAM, M D, I M S,
Director, Pasteur Institute of India, Kasauli,

M J NICHOLAS, I M D,
Assistant to the Director,

AND

B N LAHIRI, I M D

(Investigation aided by the Indian Research Fund Association)

THE action of ether on the rabies virus is now a matter of considerable importance owing to the introduction of an etherized vaccine for treatment purposes

THURS DEC. 8 10 A M 1 P M

According to Remlinger (1919) Roux was the first to test the effect of ether on the rabies virus Later, Remlinger himself, and later still Alivisatos (1922) and Hempt experimented with this method of attenuation Remlinger showed that, in rabbits and guinea pigs, fixed virus remained alive after immersion for 53 hours, if taken from the superficial parts of the brain and for 120 hours, if obtained from the deeper parts Alivisatos, experimenting with dogs and sheep, showed that the incubation period in dogs was lengthened from 10 to 18 days by immersion of the brain for from 48 to 96 hours After 120 hours viability was uncertain and, after 140 hours the virus was dead In cords, the virus was destroyed in a few hours He reported similar results with street virus

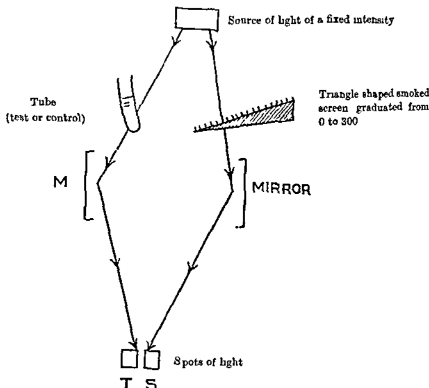
The European observers are thus in agreement on this question

In India Cornwall (1926) has shown that the fixed virus in the Pasteur Institute of Southern India was dead after immersion for 48 hours The fixed virus at the Coonoor Institute is a descendant of the present Kasauli fixed virus.

The present authors (1926 27) working with the Kasauli fixed virus showed that in infected brains marked attenuation occurred at 36 hours and the virus was usually dead after 84 hours' immersion In infected cords, the virus was killed in

least difference which occurs between the optical densities of the test tube and the control tube

The light at S is made variable by means of the screen which permits the intensities to be equalized and thus allows of a measurement with T by means of a graduation of the screen



For example, we take a reading on the test tube and after equalization we see 250 on the screen On the control tube, we find 160, 250—160—90, which is the syphili metric index This means the measurement of the intensity of the flocculation which has occurred, which is proportional to the infection

If the same number is found in the two readings, the difference (zero) means no flocculation and thus no infection

RABIES AND ANTI RABIC TREATMENT

THE ACTION OF ETHER ON THE RABIES VIRUS

BY

LIEUT COL J CUNNINGHAM M D I M S
Director Pasteur Institute of India Kasauli

M J NICHOLAS I M D
Assistant to the Director

AND

B N LAHIRI I M D

(Investigation aided by the Indian Research Fund Association)

THE action of ether on the rabies virus is now a matter of considerable importance owing to the introduction of an etherized vaccine for treatment purposes

According to Remlinger (1919) Roux was the first to test the effect of ether on the rabies virus Later Remlinger himself and later still Alivisatos (1922) and Hempt experimented with this method of attenuation Remlinger showed that in rabbits and guinea pigs fixed virus remained alive after immersion for 53 hours if taken from the superficial parts of the brain and for 120 hours if obtained from the deeper parts Alivisatos experimenting with dogs and sheep showed that the incubation period in dogs was lengthened from 10 to 18 days by immersion of the brain for from 48 to 96 hours After 120 hours viability was uncertain and after 140 hours the virus was dead In cords the virus was destroyed in a few hours He reported similar results with street virus

The European observers are thus in agreement on this question

In India Cornwall (1926) has shown that the fixed virus in the Pasteur Institute of Southern India was dead after immersion for 48 hours The fixed virus at the Coonoor Institute is a descendant of the present Kasauli fixed virus

Th... sauli fixed virus showed ... hours and the virus was ...s the virus was killed in

THUI Dec. 10 A 1 P

less than 12 hours Working with street virus, they found that these periods we extended to 72 and from 120 to 144 hours respectively

The results obtained with the Indian fixed virus thus appear to differ consi erably from those already reported in Europe while the results with the street vir agree more or less with the European figures

Further investigation thus appeared desirable with a view to comparing tl *death* points of different strains of virus and more particularly the European ar Indian strains of fixed virus under similar conditions of experiment

The present paper records the results of this investigation

Two further strains of fixed virus have now been tested by us —

(1) The virus fixé at present in use at the Pasteur Institute in Paris kindl sent to us by M Calmette and brought to India by Col Macki I M S This virus is the strain originally fixed by M Pasteur

(2) A new strain of fixed virus fixed by us in Kasauli especially for th purpose

The technique followed in these experiments differs in no way from that alread recorded by us in our previous papers A detailed description is therefore unneces sary In each case, a series of whole brain and cords was immersed in ether fo varying periods After removal samples from the outer and innermost portions *and a mixture from the whole brain*, were taken emulsified to a strength of *l* per cent and 0 2 c c delivered subdurally into a rabbit The photos show the method adopted by us for immersing the brains in ether and storing them for use (Plate XVIII figs 1 and 2)

Our results together with those obtained with the strains of street virus and Kasauli fixed virus previously tested by us, are given in the following tables —

TABLE I

Showing the characteristics of the four strains of rabies virus tested

Name	Type	Approximate Sub passage	Sub passage period to 1st Symptoms	Death
			Days.	Days.
Indian	Street	1	12	18
Kasauli No 1	Fixed	850	7	8/9
Kasauli No 2	Fixed	38	7	9
Pasteur Institute Paris	Fixed	1 301	–	8/9

PLATE XVIII



Fig 1

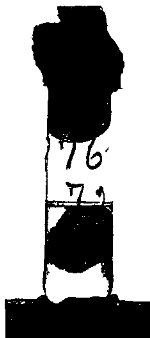


Fig 2

Fig 1 Method of immersing brains in ether

The brain is suspended in mosquito netting from a hooked glass rod which passes through the cork and by means of which the level of the brain in the fluid can be regulated Equal exposure on all sides and equal penetration is thus ensured The fluid which exudes from the brain substance can be seen at the bottom of the bottl

Fig 2 Half brain stored in 50 per cent glycerin and water until used

TABLE II

Showing the effect of immersion in ether of brains infected wit[h the four] strains of rabies virus

Name	Type		HOURS OF IMMERSION AND RESU[LTS] IN DIFFERENT SITUATIONS		
			Outside	Inside	Mi[xture]
Indian . .	Street	Alive Dead	24 48	84 96	7
Kasauli No 1	Fixed	Alive Dead			24 36
Kasauli No 2	Fixed	Alive Dead	48 72	84 96	72 84
Pasteur Institute, Paris	Fixed	Alive Dead	96 120	144 168	144 168

TABLE III

Showing the effect of immersion in ether of cords infected with the four strains of rabies virus

Name	Type		HOURS OF IMMERSION AND RESULTS IN DIFFERENT SITUATIONS		
			Outside	Inside	Mixture
Indian	Street	Alive Dead	24 48	72 84	84 96
Kasauli No 1	Fixed	Alive Dead		Less than	12
Kasauli No 2	Fixed	Alive Dead	48 72	84 96	72 84
Pasteur Institute, Paris	Fixed	Alive	84		

Table I —Shows the approximate sub passage at the time of experiment and the usual periods between subdural inoculation, first symptoms, and death, with the four strains of virus

The 'Paris' virus was in its 1,301st sub passage, the Kasauli virus No 1 in its 859th sub passage, and Kasauli No 2 in its 38th sub passage The strains of fixed virus show no material differences as to incubation period and time of death after inoculation

Table II —Gives the approximate death points of the four strains in various parts of the brain

Reference has already been made to the figures for the Indian street virus and Kasauli No 1 fixed virus

Kasauli No 2 virus is undoubtedly more resistant than Kasauli No 1, in that the mixture was alive at the end of 72 hours' immersion as opposed to 24 hours It approaches more nearly to the Indian street virus but is apparently rather more resistant if the results obtained with the outside sample in each case be accepted

The fixed virus from the Pasteur Institute in Paris is far more resistant to the action of ether than any of the Indian strains Even in the outside of the brain the virus was alive after 96 hours' immersion while a period between 144 and 168 hours was required to kill it In other words our results with this virus practically agree with those already published in Europe

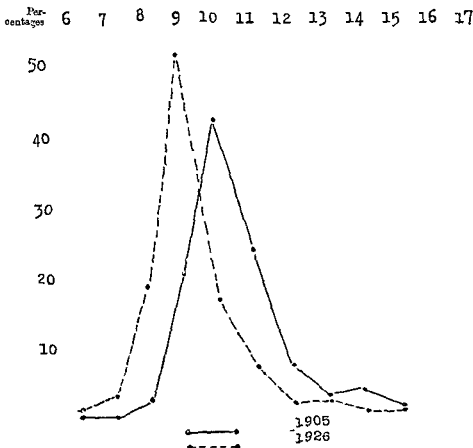
Infected cords gave very similar results to those just mentioned for the brains with the exception that death of the virus occurs rather earlier in each case

These figures clearly show that the resistance of the virus is an innate property of the virus itself and not merely due to the amount of nerve substance to be penetrated

These experiments prove definitely that different strains of virus vary considerably in regard to their resistance to ether and show that if the ether method of attenuating the virus for treatment purposes is generally adopted, this factor requires careful consideration

The real significance of these variations in different strains of virus is difficult to estimate Can the want of resistance on the part of Kasauli fixed virus No 1, for instance, be considered as a degenerative change due possibly to the age of the virus ? There is little evidence in support of such a claim In the first place, age by itself need not produce such a lowering of resistance, for, the Paris virus is older than the Kasauli virus and is much more robust than any of the Indian strains Secondly the only change which can be shown between Kasauli virus No (1), as it is at present and as it was when first fixed, points to an increase, not a loss of virulence in that the period between inoculation and death in routine sub passages has been definitely decreased (*see* Chart below) Further, the fresh strains of Indian virus tested both fixed and street, although somewhat more resistant than the older Kasauli virus, also show the same want of resistance if compared with

Paris virus It would appear from the evidence before us, therefore, that want of resistance to ether, by itself, cannot be taken as evidence of degeneration.



Showing the alteration in virulence of the 'Kasauli fixed virus' by repeated passages The curves give the percentage of rabbits dying from the 6th to the 16th day after subdural infection, in 1905, between the 24th and 96th passages (total 413 rabbits), and in 1926, about the 880th passage (total 369 rabbits)

The question of a possible correlation between the resistance of any given virus to ether and its antigenic properties is of much more practical importance No evidence is at present available on this point but comparative experiments with

the 'Kasauli' and 'Paris' viruses are at present in train at the Pasteur Institute, Kasauli, and it is hoped that information on this point will be available in the near future

REFERENCES

REMLINGER (1919) — *Annal Inst Pasteur*, Vol XXXIII p 616

ALIVISATOS, G P (192?) — Immunization against rabies with fixed virus treated with ether *Deut Med Woch*, No 2 March 3

CORNWALL (1926) — *Ind Jour Med Res* Vol XIII, No 4 April p 807

CUNNINGHAM NICHOLAS and LAHIRI (1926) — *An investigation into the value of an etherized vaccine* in the prophylactic treatment of rabies Part I *Ibid*, Vol XIV, No 2 Oct

Idem (1927) — An investigation into the value of an etherized vaccine *in the prophylactic treatment of rabies Part II Ibid*, Vol XV, No 1 July

DISCUSSION

Major A Parker Hitchens (Philippine Islands) May I ask Col Cunningham if there is evidence to show that virus used before its infectivity for dogs has been completely destroyed by ether would really be harmful if injected into human beings Is it likely that the virus after several hundred consecutive passages through rabbits has lost its human species infectivity to such an extent that it can be injected into man with impunity even though it had not lost its ability to infect rabbits

Lieut-Col J Cunningham, I M S (B India) replied The harmlessness of *fixed* virus to man is the basis of Pasteur s original method and of many subsequent methods Specific cases are known where experimenters have inoculated the contents of a whole fixed virus brain into themselves without harm resulting This loss of infectivity by the cutaneous route although practically so is not quite absolute

PHARMACOLOGY

EPHEDRINE A REVIEW OF MORE RECENT BOTANICAL RESEARCHES ALKALOIDAL CONTENT OF THE CRUDE DRUG AND EXPERIMENTS WITH EPHEDRINE AND PSEUDO EPHEDRINE TO ELUCIDATE THEIR ACTION

BY

BERNARD E READ
Professor and Head of Department of Pharmacology Pekin Union Medical College China

Introductory Note —EPHEDRINE is an adrenalin like alkaloid occurring in various species of the plant genus Ephedra together with its isomer Pseudo ephedrine It may be considered to be the chief active principle of the Chinese drug Ma Huang responsible for the claims set forth in ancient Chinese medicine for the past 5 000 years for its value in all kinds of respiratory disorders As recently as the Ming dynasty it was used as tribute to the throne from those provinces where it naturally grows and it has from the earliest times been held in great repute all over China

The introduction of this alkaloid into scientific medicine, especially for the treatment of asthma and hay fever has called for the most exhaustive researches into the botany of the crude drug the chemistry of its alkaloids its physiological action and its general pharmacology

Botanical Sources —The world s chief source of ephedrine is the crude Chinese drug Ma Huang The botanical identification of this drug has absorbed our attention for the past four years On account of its growth in the wilds in high mountainous areas, on account of the insignificant appearance of its flowers and on account of the male and female plants being separate complete identification has been delayed for two years With the extensive and intensive field studies of J C Liu for the first time we are now able publicly to announce that the commonest source of ephedrine is the species *Ephedra sinica* (Plate XX fig 2) This is an entirely new species examined and so named by the world s authority upon ephedra Dr Otto Stapf There is a second species *Ephedra equisitina* Bunge Plate XX, fig 3, which also occurs in abundance in North China

Alkaloidal Content of the Plants—The first mentioned species *Ephedra sinica* on account of its herbaceous character most commonly occurs upon the market The latter species being woody is disliked by the drug dealers Chemical analyses of these two species show that *Ephedra sinica* collected in the autumn commonly yields about 1 3 per cent of alkaloid *Ephedra equisitina* gives a slightly better *yield of about 1 6 per cent* These figures quoted represent the maximum yield in autumnal conditions We have made careful monthly analyses and find that in the spring and early summer the alkaloidal content is very low

Two Active Principles—The alkaloidal content represents the sum total of the two alkaloids which are present Chou has reported that *Ephedra sinica* has *about 80 per cent of these alkaloids in the form of* ephedrine *and about* 20 per cent as pseudo ephedrine Recent analyses by Feng of *Ephedra equisitina* show that it contains a larger amount of ephedrine both absolute and relative, of the total alkaloids about 85 per cent is ephedrine

Feng has modified the ' biuret reaction ' so that he is able to measure quantitatively *by colorimetric means any pure solution of ephedrine, also to measure* accurately any mixture of ephedrine and pseudo ephedrine to show the percentage of either present in the mixture

Part of the Plant Used—Analyses of the various parts of the plant show that there is no ephedrine in the root or in the seed, the plant has no leaves only insignificant tiny bracts occur at the joints of the stems The alkaloid resides in the stem However, separate analyses of the joints show that they contain a higher percentage of pseudo ephedrine which may explain why old Chinese medical books order the joints to be discarded

Physiological Action of Ephedrine—Ephedrine is now well known as a sympatho mimetic substance producing affects similar to those obtained from adrenalin. It produces a rather lasting rise of blood pressure due mainly to vaso-constriction There is dilatation of the bronchi and mydriasis after local or systemic administration *Applied locally or taken by mouth it reduces the* swelling of the turbinate bodies and diminishes hyperæmia

In attempting to further elucidate these affects, we have conducted numerous experiments which go to show that after paralysis with ergotoxine, ephedrine, unlike adrenalin, produces no vasomotor reversal reaction Moreover, administered *after cocaine there is no synergism as is seen with adrenalin* These experiments fully reported elsewhere go to show that whilst ephedrine is neuro tropic it is not as much so as adrenalin, nor is its action upon the sympathetic nervous system identical with that of adrenalin

Action upon the Nose—The unusual value of ephedrine in the treatment of hay fever, acute coryza, and almost any nasal condition relieved by shrinkage has led us to make a detailed comparison of its action with cocaine and adrenalin Pak and King elaborated a technique whereby the volume of the nasal cavity could be accurately measured by a *regular tambour tracing upon a drum* Large dogs anæsthetized with luminal were so prepared and results show that irrespective of

the path of administration ephedrine could produce a shrinkage of the tissues with a large subsequent increase in nasal volume far superior to either of the drugs mentioned superior in that its affects were sustained over much longer periods of time A slight degree of irritation has been reported by some workers after the use of ephedrine in bad hay fever cases Our experiments go to show that even oral administration is effective and in any case the local application of solutions stronger than 2 per cent is not advisable The addition of 0 5 per cent potassium sulphate in more than one clinical case removed the reflex irritation caused by strong solutions

Stimulation of the Central Nervous System —It has been noted that ephedrine is able to awaken dogs well anæsthetized by chloral We do not find that it has any such action upon luminalized dogs However Kreitmar has done work showing its usefulness as an antidote to scopolamine poisoning It does not inhibit the narcotic action of this drug so he recommends its use with scopolamine and morphine to prevent intoxication by these drugs and to sustain blood pressure

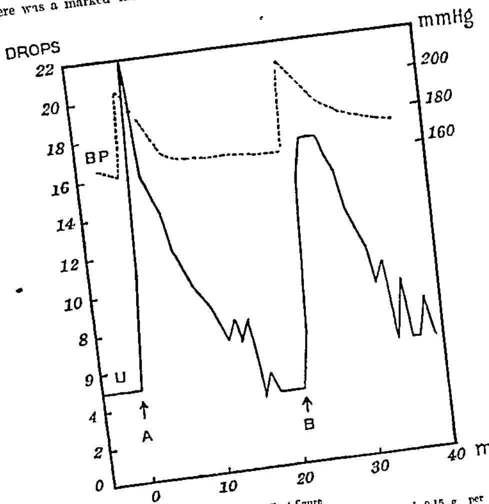
We have studied some of its reactions towards morphine and with the scattered evidence at our disposal believe that it may serve as a good antidote to acute morphine poisoning First of all experiments were made upon temperature Rabbits treated with toxic doses of morphine show the customary fall in body temperature This in every case is affected by ephedrine which raises the body temperature to normal Numerous experiments upon the human show that half grain doses will usually produce an increase in body temperature ranging from 0 4 to 0 7 degrees centigrade This affect upon temperature is directly related to the sugar metabolism Estimations of the blood sugar show a marked rise especially during the first hour after injection of the drug which gradually returns to normal in about three hours After morphia ephedrine still produces its stimulating affect upon the respiration and with its affect on the blood pressure in producing a long sustained rise it holds out possibility of being of value superior to caffeine after acute morphine poisoning

Pseudo ephedrine

Much work has been done upon ephedrine and very little upon its isomer pseudo ephedrine it being briefly dismissed by such phrases as the affects of pseudo ephedrine are apparently very similar to ephedrine We have shown that whilst the action of pseudo ephedrine upon blood pressure is only about half as intense as that of ephedrine its basic character is different and altogether it simulates the action of pituitary In similar experiments to those conducted with ephedrine, with ergotoxine and cocaine we have shown that its action is chiefly musculo tropic and it may have some stimulating action on the para sympathetic system

Unlike ephedrine it does not contract the blood vessels With this in mind we have just completed a series of experiments to test out the possible diuretic affects of pseudo ephedrine Fourteen successive experiments upon dogs all gave the same kind of result The dogs were given plenty of fluid the day previously

and were luminalized for experiment Intravenous injection of half of one milligram of the drug per kilogram body weight produced an increase in the flow of urine A typical experiment is shown in Plate XIX, fig 1, where even after the blood pressure had returned to normal and had further fallen to sub normal there was a marked increase in the output of urine Text figure below shows



Text figure

Experiment 25th July, 1927 Dog (male) 11 3 kilos Luminal 0 15 g per
subcutaneously Five hundred c cs of water were given per os one day previou
experiment and 500 c cs of water per os just before the anæsthetic Three hun
of saline were injected intravenously 30 minutes before the first injection of drug
by C Pak and B E Read

U Drops of urine
B P Carotid blood pressure
A 5 5 mg pseudo-ephedrine intravenously
... do ephedrine intravenously

PLATE XIX

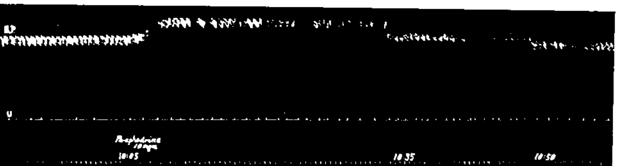


Fig 1

Pseudo ephedrine Diuresis
Carotid blood-pressure (B P), Urine drops (U)
Experiment by C Pak and B. E. Read.

quite plainly the dramatic affect obtained in another experiment After a corresponding to about half a grain in the human there was an increase of flow from 5 drops to 22 drops per minute, which was repeated 20 minutes with a further dose which yielded a rise from 4 to 17 drops

The most striking thing about most of these experiments was that the di continued irrespective of the lowering of the blood pressure

It is scarcely necessary to point out how promising this is for the treat of cases of ascites with high blood pressure It is possibly one more to be a to the small group of drugs at the physician's disposal for dealing with most troublesome class of disease

Ephetonin

Mercks have manufactured a synthetic ephedrine called ephetonin wh actually the optically inactive isomer of ephedrine They state that its is identical to that of ephedrine We should like to note briefly here tha experiments show this isomer not to be identical in action but it stands be ephedrine and pseudo ephedrine in its neuro tropic and its musculo tropic affe

DISCUSSION

Dr S Kubota (Kwantung) Since Dr Nagai, a Japanese chemist, first is ephedrine from a Chinese drug, Ma Huang (*Ephedra vulgaris*) it is now nearly l century Fifteen years ago taking up ephedrine as a subject of our pharmacol study we could experimentally show that the action of it is very much like tl adrenalin and made suggestions that it could be used as an internal curative for as but for ten years after that there was no enthusiasm about it In these few since Professor Read and his co workers in Peking published a lot of valuable wo ephedrine it attracted attention and now several drugs containing natural or sy ephedrine, are introduced into the therapeutic world Though it does not strongly as adrenalin it is considered to be more advantageous in that it can be internally and the action may last longer than adrenalin

A few years ago I was on a tour in inner Mongolia on a medical expedition I see ephedra growing everywhere we passed Later we used it as material for our but the amount of ephedrine contained in it is not as big as that of India of Lieut Col Chopra told me the day before yesterday

It is written in Pen Tsao Kwan Mu, a famous Chinese materia medica that th of ephedra has an action contrary to that of the leaf As is known, the leaf of ep containing ephedrine, causes a rise of blood pressure but if we try the extract fro root it lowers blood pressure and we tried to isolate the blood pressure lowering Several times we could see crystals of needle form but the isolation was not succ It easily turns to a substance which causes a rise of blood pressure, probably ephe so it is believed that the root may contain a body which can be turned to ephedr some biological process

Lieut Col R N Chopra, I M S (Bengal) Said he was very interested in the by Dr Read [illegible]

wanted to draw attention Firstly, there were three varieties of ephedrine growing in India two of these varieties i e *E vulgaris* and *E intermedia* he had analysed He found that they gave a good yield of total alkaloids 80 per cent of which was ephedrine He further gave results of his clinical experience with the drug In cases of asthma the drug had a marked beneficial action in controlling the paroxysms as well as relieving the attacks He also tried the drug in a number of cases of ascites but the diuretic effect produced was not marked

A RETROSPECT OF SIX YEARS' RESEARCH WORK ON THE INDIAN INDIGENOUS DRUGS

BY

LIEUT COL R N CHOPRA M A M D (Cantab), I M S

Department of Pharmacology Calcutta School of Tropical Medicine and Hygiene

ALTHOUGH the study of Indian indigenous drugs was begun nearly a century ago it was at first mainly confined to the collection of available information about various medicinal plants growing in this country A number of books was published which contain information from Ayurvedic (Hindu medicine) and Tibbi (Mohammedan medicine) sources and in some instances the writers had given the results of their personal observations and experiences Warden and Hooper were the first to carry out a laborious study of the chemical composition of the important medicinal plants growing in India The Indigenous Drug Committee (now abolished) made standard preparations of some of these remedies and issued them to various Government hospitals and dispensaries for clinical trials but no very useful information was obtained Attempts have also been made by individual workers from time to time to investigate the pharmacological action of drugs by modern methods of research, but these workers were seriously handicapped for want of properly equipped laboratories The result was that the pharmacology of most of the remedies used in the indigenous medicine remained practically an unexplored field

Six years ago the Calcutta School of Tropical Medicine and Hygiene was opened and a chair of experimental pharmacology was established for the first time in the history of medicine in India One of the duties allotted to the professor of pharmacology was to make a thorough study of the drugs growing in this country, to analyse them chemically isolate their active principles and work out their pharmacological action where this had not been already done A careful survey of all the Indian medicinal plants was taken and two aspects the problem forcibly presented themselves from the scientific as well as from the economic points of view

Firstly there was a large group of drugs that are of known value and were recognized by the British and other pharmacopœias A large number of these medicinal plants grow wild and in great abundance in many parts of India and a certain number are even cultivated The soil the season and the gathering time are some of the important factors with plants and it can hardly be expected that the amount of active constituents would be constant under all conditions In the

alkaloid ephedrine It has been said that the total alkaloidal content and the proportions of ephedrine and pseudo ephedrine vary according to the locality in which it grows *E vulgaris* Rich grows abundantly in the drier regions of the temperate and Alpine Himalayas from Western Tibet to Sikkim ascending to a height of 16 000 feet Large quantities can be obtained in the Shalai hills north of Simla at an altitude of 10 000 feet Analysis of a specimen obtained from that source gave the total alkaloidal content to be 0 92 to 1 22 per cent of which 80 per cent was ephedrine and 20 per cent pseudo ephedrine This compares favourably with Chinese *E vulgaris* which yields from 0 2 to 0 9 per cent of total alkaloids *E intermedia* is also rich in ephedrine

Secondly we have been investigating some of the well known remedies that are used in the indigenous systems of medicine both Hindu or the Ayurvedic and Mohammedan or the Tibbi systems It is believed that out of a large number of drugs used in the indigenous systems of medicine for centuries past and still in use there must be at least some that might deserve the reputation they have earned as cures History shows that many of our important pharmacopœial drugs were known and were used in some form or other long before they were introduced into our medicine and before their actions were investigated On the other hand there are sure to be a large number of drugs of little therapeutic value that are used only because they are mentioned in some of the old works and no one has taken the trouble to confirm the truth of these statements An attempt must be made to separate the good ones from the useless ones A systematic investigation of these drugs is now in hand We are gradually analysing them with a view to finding out their active principles We are determining their pharmacological action and are trying them clinically to see whether the claims made regarding their efficacy can be proved or disproved It is only by a careful pharmacological and clinical study that the action of these drugs and their active principles can be established and they can be made available for the alleviation of human sufferings

The first of this class of drugs that we investigated was *Bœrrhavia diffusa* which belongs to the natural order Nyctagineæ It is commonly known in the vernacular as Punarnava and has been largely used from time immemorial as a diuretic by the Hindu physicians This plant grows all over India as a common creeping troublesome weed and is specially abundant during the rains There are several varieties of it but the white flowered variety is considered to be the best for medicinal purposes *Chemical analysis showed that the active principle is a* body of alkaloidal nature besides this large quantities of potassium salts, especially potassium nitrate are also present Intravenous injections of the alkaloid in animals produced a slight but persistent rise of blood pressure and a well marked diuresis chiefly due to its action on the renal epithelium *Clinically* a liquid extract made from either the dry or fresh plant produced marked diuresis in cases of œdema and ascites

The presence of a large amount of potassium salts in the extract undoubtedly reinforces the action of the alkaloid We found the drug useful in cases of ascites

due to early liver peritoneal (Hale White type) and kidney conditions A marked and persistent diuresis was produced the urine in some cases increasing to 120 to 130 ounces a day and the ascites entirely disappearing in some cases

Cephalandra indica is a perennial creeping herb belonging to the natural order Cucurbitaceæ and grows abundantly throughout India in a wild state It is known by the name of ' vimba ' in Sanskrit Kunderi ki bel in Hindi In Hindu medicine the juice of the plant is recommended in the treatment of diabetes and many physicians practising the western system in this country testified to its sugar reducing qualities in this disease We made a chemical analysis of the plant but found no very active constituents We also searched for bodies of the nature of hormones and enzymes as Dubbins and Corbett (1923) showed that certain plants and vegetables contain both the sugar reducing and blood sugar increasing principles and Collip (1923) isolated glucokenin from plants which when injected reduce the blood sugar No substances of this nature were found in *Cephalandra indica* and administration of the fresh drug as well as preparations made from it administered by the mouth or by subcutaneous injection had no effect whatever on the blood sugar or sugar in the urine of animals or human subjects

Butea frondosa (Polas) grows extensively in the plains of India and has been used for expelling intestinal parasites by the Hindu physicians Fresh seeds ground in form of a powder when tried in a large series of patients suffering from ascaris infection gave variable results In one series of over 30 cases the drug proved to be nearly as efficacious as santonin in expelling ascaris In another similar series it did not prove to be so effective but the seeds were old and worm eaten The drug had no appreciable effect on any of the other helminths Chemical analysis of the seeds showed 16 per cent of a fixed oil and a resin but we were unable to isolate the fraction in which the anthelmintic property resided Our further experience proves that powdered seeds have an undoubted action in expelling round worms but they are difficult to take as they often produce nausea and vomiting

Terminalia arjuna (arjun) is a well known cardiac tonic in Hindu medicine The drug contains a body of glucosidal nature but we did not find it possessed any very marked stimulant effect on the heart

Silajit is an exudation from rock surface obtained during the hot weather months in the lower Himalayan hills It is an important drug of the ancient Hindu materia medica and is extensively used by the Hindu physicians in a variety of conditions It is said to be particularly effective in pulmonary diseases such as phthisis bronchitis asthma It is specially recommended in diabetes Chemical analysis showed that besides gum albuminoids traces of resin and fatty acids it contains large quantities of benzoic acid and hippuric acids and their salts The benefits ascribed to the drug in respiratory diseases are undoubtedly due to the presence of these compounds In diabetes however we found that the administration of this drug by the mouth had no effect either on the blood sugar content or the urine sugar content

Adhatoda vasica is a large shrub which grows wild and abundantly in almost every part of India The plant has a reputation all over the country as an expectorant and anti spasmodic and is commonly used as a remedy for tuberculosis of the lungs and other chest affections It is so much valued in this disease by the Hindu physicians that there is a popular saying that where this plant exists no man suffering from phthisis need despair for long Many preparations of this drug are used and so strong was the belief in its efficacy even among those practising western medicine that it found its way into the addendum to the British pharmacopœia The drug was chemically analysed as early as 1888 by a Dutch chemist and an alkaloid called vasicine and an essential oil were isolated We worked out the pharmacological action of the alkaloid and tried preparations made from the plant in tuberculosis of lungs Although it has undoubted expectorant properties and liquefies the sputum which is coughed up more readily and although it has a sedative and a broncho dilator effect we did not find the drug had any action in pulmonary tuberculosis The alkaloid did not have any inhibitory effect on the growth of tubercle bacilli in cultures

Vitex peduncularis is a plant belonging to the natural order *Verbbenaceæ* and is commonly known in Hindi as Nagbail It grows abundantly in Eastern Bengal Bihar and Central Provinces The aboriginal tribes of these parts believe it to have curative properties against malaria blackwater fever and kala azar Vaughan tried it in the form of a decoction made by boiling the leaves in water and obtained excellent results from its use in malaria The reputation of the drug spread and a few years ago orders were received from America and other parts of the world for supply of large quantities of the drug We obtained authentic species of the plant analysed them and found that no active principles of the nature of a glucoside or alkaloid could be detected in it We carefully tried the drug in a series of cases of malarial fever and found that it had no effect whatever either on the asexual or the sexual forms of any of the *P vivax P malariæ* and *L malariæ* In fact two of our cases of sub tertian malaria began to show cerebral symptoms Administration of quinine in all these controlled the symptoms and the parasites disappeared from the peripheral blood Hodgson showed that the drug has no effect whatever in kala azar

Saussurea lappa (Kuth root) grows in the north western portion of the Himalayas especially on the moist slopes of the mountains round the valley of Kashmir It is known by the name of *Kusht* in Sanskrit and '*Kuth*' '*Kusht*' or *Patchak*' in Hindi The root of this plant has a peculiar strong aromatic fragrant smell which resembles that of the orris root It has been employed in Hindu medicine for many centuries and enormous quantities of the root are also exported to China every year where it is used as an incense and is also said to have found its way into the Chinese medicine Analysis of the root showed that it contains 0 86 per cent of a yellowish looking essential oil which is responsible for the odour of the root it also contains traces of a glucoside and an alkaloid but these are not very active The drug is an excellent remedy in spasmodic conditions of the respiratory

tract, it stimulates the secretion of mucus and has a liquefying effect on it when it is tenacious We have used an alcoholic extract prepared from the root with great benefit in the vagotonic type of asthma which is the prevalent form in India In these patients the action of the vagus is increased owing to certain causes producing spasm of the bronchial musculature and vaso-dilatation of the bronchial mucosa, which gives rise to asthmatic attacks The effect can be relieved by giving atropine which diminishes the vagus action or adrenalin, which stimulates the antagonistic action of the sympathetic Both these drugs have obvious disadvantages Kuth root extract not only decreases the vagal but at the same time relieves the congestion of the bronchial mucosa by its expectorant action, which is absent in case of the other drugs We have used this extract for the last three years in treatment of asthma with excellent results Not only are the attacks cut short but their frequency is also reduced A drachm of the extract administered to a patient when he gets the premonitions of the attack will usually stop it

Holarrhena antidysenterica is a small deciduous tree with white flowers belonging to the natural order *Apocynaceæ* The plant is known in Sanskrit as *Kutaja* ' and in the vernacular it is called *Kurchi* (Bengalee) or *Dudhi* (Hindi) It is a native of the tropical Himalayas growing up to a height of 3 500 feet above the sea level It also grows abundantly throughout the dry forests of India even as far south as Travancore The bark of this plant has been used in the indigenous medicine against dysentery and different observers were so struck by its beneficial effects in this disease that it was advocated that it should be included in the British pharmacopœia Although the chemistry of *H congolensis* an allied species growing in Africa, has been worked out the composition of the Indian bark was not been thoroughly investigated We found it contains three alkaloids of which conessine is well known while the other two which resemble conessine in action occur in fairly large quantities We have shown that conessine has a remarkable effect on *E histolytica* in vitro It kills these organisms in such high dilutions as 1 in 280 000 in alkaline solution as compared with 1 in 200,000 of emetine under similar conditions In view of these findings it is hoped that the drug may be of great value in the treatment of amœbic dysentery and it is being clinically tried Though the clinical data collected so far are not sufficient to draw any definite conclusions, injections of conessine hydrochloride or tartrate appear to be well borne and have a curative value in amœbic dysentery which is equal to that of emetine Further work is in progress

Psoralia corylifolia is a common herbaceous weed belonging to the natural order Leguminosæ which grows throughout the whole length and breadth of the plains of India It is called ' *Vakuchi* ' in Sanskrit and *Bukahi* ' or ' *Babchi* ' in Hindi The seeds of this plant have been used in Hindu medicine for a long time in treatment of leprosy and so effective is it considered in this disease that it has been given the name of ' *Kusht nashini* ' or leprosy destroyer It appears that the term ' *Kusht* ' or Leprosy is rather loosely used by the Hindu physicians, such conditions as leucoderma, psoriasis chronic streptococcal dermatitis being all included under

this heading The drug is given internally by the mouth and is also applied locally to the lesions The active principle of the seeds is an essential oil which has a powerful effect against the skin streptococci and there are also quantities of a resin An oleo resinous extract of the seeds containing most of the essential oil and the resin has been largely used by Col Acton in the treatment of leucoderma with *beneficial results especially when the disease is of non syphilitic origin* The essential oil appears to have a specific effect on the arterioles of the sub capillary plexuses of the skin which are dilated by it so that the plasma in the area is increased The skin becomes red its nutrition is generally improved, the melanoblasts are stimulated leading to the formation of pigment which is exuded and diffuses into the decolorized leucodermic patches

These are examples of some of the drugs we have investigated and such are the lines on which we are conducting this inquiry The examination of the first group of drugs that is those known to the pharmacopœias and their allied plants which could be used as substitutes for pharmacopœial drug is not difficult and requires little skill *It is however important from the economic point of view and it is a matter of great* satisfaction to see that the work is already bearing fruit and the drugs grown in India are being utilized more and more The investigation of the second group of drugs that is those used in the indigenous systems of medicine in this country, is a much more difficult matter Almost every common plant and shrub growing in this country has ascribed to it some medicinal property These beliefs in some cases originated from the teaching of old commentators and are based on clinical data but in others there is nothing reliable Their introduction was accidental and often a drug has come into use because a single case happened to have derived some benefit from it In this way remedies have multiplied without proof but by belief and as they hail from all parts of India no one seems to have any correct notion about their use and properties

A thorough research of these drugs would be the work of many generations of chemists and pharmacologists For practical purposes we are taking advantage of the experience of the practitioners of the old systems in vogue in guiding us as to the order of priority in which the drugs should be investigated To avoid wasting time and energy we often carry out clinical trials before handing over the drug for analysis to the chemists *It is carefully tried in a number of cases and if good* results are obtained it is proceeded with otherwise it is discarded

The time, labour and expense necessary to work out the action of a single drug is enormous An experienced chemist takes five to six months to isolate in a pure state and state the nature of the different chemical constituents of a single crude drug the isolation of a sufficient quantity of the active principles and testing them pharmacologically takes a few more months the testing of the therapeutic action of drug takes no less time *We have worked out a number of these* drugs and I must confess that although we have come across some potent remedies we have not so far discovered anything of any outstanding merit which would make a distinct advance in the treatment of any disease or condition

PEFEPENCFS

CHOPRA R N and GHO H B N (19 3)	The I eld for Pesearch in India i Indigenous Drugs *Ind Med Gaz* Vol LVIII No 3 March
CHOPRA R N GHOSH S GHOSH B N and DE P (19 3)	The Ilarmacology and Therapeut cs of *Boerhavia d ffusa* *Ib d* Vol LVII No o May
CHOPRA R N KNOWLES P and GUPTA J C (19_4)	On the Failure of *I itex peduncularis* in the Treatment of Malaria *Ibid*
CHOPRA R N and ASA C CHANDIER (19 4)	Indian Santonin *Ib d* Vol LIX No 11 Nov
CHOPRA I N and DE P (19 4)	A Preliminary Note on the Pharmacolog cal Act on and Therapeutic Propert es of Kuth Root—*Saussurea lappa* *Ib d* Vol LIX No 11 Nov
CHOPRA R N BOSE S C and DE P (19 ɔ)	Variat ons in the Potency of D g talis Preparat ons in the Tropics *Ibid* Vol LX No 3 March
CHOPRA R N and BOSE J P (19?5)	Observat ons on the Ant d abet c Properties of *Cephaland a ind a* (Telakucha)
Idem (19 ɔ)	*Cephalandra nd ca* (Telakucha) in D abetes *Ind Jour Med Res* Vol XIII No I July
CHOPRA R N and GHOSH S (19 ɔ)	A Preliminary Note on the Pharmac lo_y and Thera peutics of *Adhatoda vas ca* (Basak) *Ind M l Ga* Vol LX No 8 Au_
Idem (1 ɔ)	Some Obs rvations on the Pharmacolog cal Act on and Therapeut c Propert es of *A lhatod vas ca* (Basak) *Ind Jour M d Res* Vol XIII No ? O tober
CHOPRA I N and GHO I N N (19 6)	Some Medicinal Plants growin_ in the H malayas *Ib l* No 3 January
CHOPRA P N and DE P (19 6)	Indian Di_ talis *Ind Med Ga* Vol LXI No 3 March
CHOPRA P N POSE J I and GHOSH N N (19?6)	Chemical Compos t on and Ant diabet c Propert es of S laj t *Ind Jour Med. R s* Vol. XIV No 1 July
CHOPRA R N and DE P (19?6)	B olo_ cal Assay of Dig talis Preparations in the Trop cs *Ibid* Vol XIII April
Idem (19?6)	Variat ons in the Potency of D g talis Preparations in the Trop cs *Ind Med Gaz* Vol LXI May
CHOPRA R N GUPTA J C DAVID J C and GHOSH S (19?7)	Observat ons on the pharmacological a t on of conessine The Alkalo d of *Holarrhena antidysenterica* *Ibid* Vol LXII March
CHOPRA P N and CHATTERJEE N R (19?7)	*Psoral a coryl fol a* (Babchi) Its const tuents pharmaco lo_ cal act on and therapeut c propert es *Ind Jour Med Res* Vol XV July

DISCUSSION

Dr J W Tor b (Bihar and Orissa) Aske l Col Chopra if any investigation had as yet been made of the effect of conessine hydrochloride on the muscle of the heart

Dr F Maja Das (United Provinces) Asked Col Chopra whether the plant (a vegetable parasite growing on the *nim* tree) called *gillo* or *guruch* had any anti malarial properties It was very widely used in North India combined with *chiratta* and black pepper

Dr S Subba Rao (Mysore State, B. India) · Said he was very anxious to hear Col Chopra's experiences regarding the use of ephedrine in bronchial asthma but he did not say much about it, evidently on account of the fact that the previous paper dealt with this subject He had used ephedrine in four bad cases of asthma with almost magical results The drug had to be used with great caution, as there was not much known about its toxic effects As the drug was used frequently in bad cases of asthma with frequent attacks, he should be much obliged if Col Chopra could tell him whether ephedrine had any cumulative effect, and, if so, what were its toxic symptoms on the appearance of which the drug should be stopped

Dr K P Basu (Assam) Said that in his experience as civil surgeon of the Goalpara District in 1926, in 12 cases of blackwater fever which were treated with the infusion of *Vitex peduncularis* 1 in 20, about 20 ounces in 24 hours, the parasites persisted even after 80 ounces of the infusion had been given In 1927 in the Sadya frontier tract in one case of blackwater fever, who received 120 ounces of the infusion, the temperature persisted and the parasites were detected, though scanty, up to the fifth day and yielded only to quinine

Dr B N Vyas (United Provinces) A question has just been asked as to the efficacy of *gillo*, a leafy parasitic creeper, found, I believe, in most parts of this country I do not know if Col Chopra has tried it and what results he arrived at, but I have tried this drug clinically in my department in King George's Hospital, Lucknow, and I must say the results were disappointing At times it did seem to be good in certain fevers of undefined nature but in properly diagnosed malarial fever it did not have any appreciable effect The drug is intensely bitter, has possibly a certain amount of bitter tonic action, but beyond that I doubt if it has much action though it has a great reputation as an anti malarial remedy in the country

Lieut Col R N Chopra I M S (Bengal) In reply said he was grateful for the suggestions of the speakers He had heard of the drug Dr Maya Das and Dr Vyas referred to but had had no personal experience of it As regards the cumulative and toxic action of ephedrine, he was not in a position to speak much about it as he had not tried it in a sufficient number of cases

OBSERVATIONS ON THE STABILITY OF CHLORIDE OF LIME STABILIZED CHLORIDE OF LIME AND PERCHLORON IN THE PLAINS OF BENGAL

BY

J WALKER TOMB O B E M A M D D P H
Medical Officer of Health Asansol Mines Board of Health

In the *Journal of the Royal Army Medical Corps* 1922 Dec Vol 39 No 6 pp 459 460 F S Aunomier and S Elliott published a Note on Stabilized Bleaching Powder for Tropical Use The method advocated was to add 20 per cent dry quicklime to ordinary bleaching powder This mixture they stated remains dry and powdery in hot climates does not corrode tins and only loses a very small proportion of its available chlorine when exposed to the atmosphere

From investigations extending over two years carried out in the laboratory of the Asansol Mines Board of Health it would appear that the first two statements may be accepted as valid such a mixture having been found to remain dry and not to corrode tins to any marked extent even during the rains in the plains of Bengal The statement however that it only loses a very small proportion of its available chlorine when exposed to the atmosphere cannot be substantiated

Three series of experiments were instituted to determine the rate of deterioration of pure and so called stabilized bleaching powder (1) in open tarred kerosine tins (2) in closely stoppered glass (pickling specimen) bottles and (3) in the author s glazed earthenware jars designed for the preservation of bleaching powder and described in the *Indian Medical Gazette* of February 1924

From the details recorded in the accompanying tables it will be noted that under all conditions of experiment unsophisticated bleaching powder suffered less deterioration than so called stabilized bleaching powder even when the former was in so liquid a state from exposure to the atmosphere during the rains as to be capable of being poured from its container

TABLE I

Rate of Deterioration of Bleaching Powder per week in open tarred tins

Period of year	Pure	Stabilized
Dry Cold Weather	1 5 per cent	2 0 per cent
Dry Hot Weather .	3 3 ,	5 0 ,,
Rains	7 0 ,,	8 0 ,,

We shall first consider the experiment in open tarred kerosine tins

During the dry cold weather the weekly average percentage deterioration of pure bleaching powder in open tarred kerosine tins (middle samples) was 1 5 per cent as compared with an average deterioration of 2 per cent in stabilized bleaching powder

During the dry hot weather, the corresponding percentages of deterioration were 3 3 per cent and 5 per cent and during the rains 7 per cent and 8 per cent respectively

TABLE II.

Rate of Deterioration of Bleaching Powder per week in glass stoppered bottles.

Period of year	Pure	Stabilized
Dry Hot Weather	1 2 per cent	1 5 per cent
Rains	3 0 ,	5 0 ,
Dry Cold Weather	1 0 ,	1 2 ,

Stored in closely stoppered glass bottles, the percentages of deterioration were respectively 1 2 per cent and 1 5 per cent during the dry hot weather, 3 per cent and 5 per cent during the rains, and 1 per cent and 1 25 per cent during the dry cold weather

TABLE III

Rate of Deterioration of Bleaching Powder per week in author's closed jars

Period of year	Pure	Stabilized
Rains	2 0 per cent	2 5 per cent
Dry Cold Weather	1 33 ,	2 0 „
Dry Hot Weather	1 75 ,	2 2ɔ

In the author's glazed earthenware jars the percentages of deterioration were respectively 2 per cent and 2 5 per cent during the rains 1 33 per cent and 2 per cent during the dry cold weather and 1 75 per cent and 2 25 per cent during the dry hot weather

TABLE IV

Rate of Deterioration of Perchloron per week

Period of year	In closed drum	In open tins
Dry Hot Weather	13 per cent	48 per cent
Rains	6 ,	2 9
Dry Cold Weather		

An investigation was also carried out into the rate of deterioration of 'Perchloron'—a stable preparation of bleaching powder manufactured in Frankfurt A M, Germany The strength of 'Perchloron' is approximately double that of ordinary bleaching powder and its rate of deterioration when preserved in its original drum, which possess a screw down lid with an asbestos ring rendering the drum practically airtight is from 10 to 25 times less than bleaching powder exposed to the atmosphere as it ordinarily is when in use in open drums or tins When stored *in its original drum with the lid tightly screwed down, the weekly rate of deteriora*tion of Perchloron is 0 6 per cent during the rains and 0 13 per cent during the dry hot weather The rate of deterioration during the dry cold weather has not yet been determined but is of necessity still lower The cost of Perchloron per cwt is approximately double that of ordinary bleaching powder, but this is at once equalized by the fact that its content of available chlorine is double that of bleaching powder Since its keeping qualities are so many times greater than that of bleaching powder even when the latter is preserved in special containers

such as the author's earthenware jars, it is obvious that Perchloron is the preparation of choice for use in future in hot countries such as India in public health work for all purposes for which bleaching powder is now ordinarily used

APPENDIX I.

BLEACHING POWDER IN OPEN TARRED TINS

Experiment No I

Table I

Result expressed as per cent of Av. Cl in the middle samples

Dry cold weather

Date	Average Monthly Absolute Humidity	Average Monthly Maximum Temperature, Far	Physical Condition	Pure	Average fall per week	Physical Condition	Stabilized	Average fall per week
					Per cent			Per cent
8th November 1926				28 80			28 80	
15th November, 1926				28 37			28 23	
22nd November, 1926	478	83 2°		27 95			27 67	
29th November 1926				27 53			27 12	
6th December, 1926				27 12			26 58	
13th December, 1926	422	78 6°		26 72	1 5		26 05	2 0
20th December, 1926				26 32			25 53	
27th December 1926			Moist powder	25 93		Powder	25 03	
3rd January, 1927				25 54			24 72	
10th January, 1927				25 16				
17th January 1927	422	76 5°		24 78				
24th January, 1927				24 41				
31st January, 1927								
7th February 1927								
14th February 1927	471	80 8°						
21st February, 1927								
28th February, 1927								

Dry hot weather.

Date	Average Monthly Absolute Humidity	Average Monthly Maximum Temperature, Far	Physical Condition	Pure	Average fall per week	Physical Condition	Stabilized	Average fall per week.
					Per cent			Per cent
28th February, 1927				22 64			20 88	
7th March, 1927				21 89			19 84	
14th March, 1927	550	89 8°		21 17			18 85	
21st March 1927				20 48			17 91	
28th March, 1927				19 81			17 01	
4th April, 1927				19 16			16 16	
11th April, 1927				18 53	3 3		15 35	5 0
18th April, 1927	788	103 7°	Moist powder	17 92		Dry powder	14 58	
25th April, 1927				17 33			13 85	
2nd May, 1927				16 76			13 16	
9th May, 1927				16 21			12 50	
16th May, 1927	893	99 1°		15 68			11 88	
23rd May, 1927				15 17			11 29	
30th May, 1927				14 68			10·73	
6th June 1927	973	97 5°		14 21			10·20	

BLEACHING POWDER IN OPEN TAPPED TINS

EXPERIMENT No II

TABLE I

Result expressed as percentage of Av Cl in the middle samples

Rains

Date	Average Monthly Absolute Humidity	Average Monthly Maximum Temperature Far	Physical Condition	Pure	Average fall per week	Physical Condition	Stabilized.	Average fall per week
					Per cent			Per cent
19th July 1927				26 6			26 6	
26th July 1927	973	89 8°		21 7			24 5	
2nd August, 1927				23 0			22 5	
9th August 1927				21 4			20 7	
16th August 1927	981	89 0°		19 9	7 0		19 0	8 0
23rd August 1927			Fluid	18 5		Moist powder	17 5	
30th August 1927				17 1			16 1	
6th September 1927				15 9			14 8	
13th September 1927				14 8			13 6	
20th September 1927	975	92 7°		13 8			12 5	
27th September 1927				12 8			11 5	
4th October 1927				11 9			10 6	
11th October 1927	803	90 8°		11 1			9 8	

BLEACHING POWDER IN GLASS STOPPERED BOTTLES

Table II

Result expressed as percentage of Av Cl in the middle samples

Dry hot weather

Date	Average Monthly Absolute Humidity	Average Monthly Maximum Temperature Far	Physical Condition	Pure	Average fall per week	Physical Condition	Stabilized	Average fall per week
					Per cent			Per cent
7th April 1926				30·10			30·10	
14th April 1926				29·74			29·65	
21st April, 1926	543	98·2°		29·38			29·20	
28th April, 1926				29·03			28·76	
5th May, 1926				28·68			28·33	
12th May 1926			Dry powder	28·34	1·2	Dry powder	27·91	1·5
19th May, 1926	864	102·1°		28·00			27·49	
26th May, 1926				27·66			27·08	
2nd June 1926				27·33			26·67	
9th June, 1926				27·00			26·27	
16th June 1926				26·68			25·88	
23rd June 1926	921	105·8°		26·36			25·49	
30th June, 1926				26·06			25·11	

Rains

Date	Average Monthly Absolute Humidity	Average Monthly Maximum Temperature, Far	Physical Condition	Pure	Average fall per week	Physical Condition	Stabilized	Average fall per week
					Per cent			Per cent
30th June, 1926				26 06	·		25 11	··
7th July, 1926				25 28			23 85	··
14th July, 1926	974	90 1°		24 52	··		22 66	··
21st July, 1926				23 78			21 53	·
28th July, 1926				23·07	··		20 45	
4th August, 1926				22 38	··		19 43	
11th August, 1926				21 71			18 40	
18th August 1926	982	88 0°		21 06	3 0		17 54	5 0
25th August, 1926				20 46			16 66	··
1st September, 1926			Moist powder	19 82		Dry powder	15 83	
8th September, 1926				19 23	·		15 04	
15th September, 1926	953	88 3°		18 65			14 29	
22nd September, 1926				18 09			13 78	
29th September 1926				17 55			13 09	
6th October 1926				17 02			12 44	
13th October, 1926				16 51			11 82	
20th October, 1926	753	89 9°		16 01			11 23	
27th October 1926				15 33			10 67	
3rd November, 1926				14 87			10 14	
10th November, 1926	478	83 2°		14 42			9 63	

Dry cold weather

Date	Average Monthly Absolute Humidity	Average Monthly Maximum Temperature Far	Physical Condition	Pure	Average fall per week	Physical Condition	Stabilized.	Average fall per week
					Per cent			Per cent
10th November, 19_6				14 42			9 63	
17th November, 19°6	478	83 2°		14 28			9 51	
24th November, 19°6				14 14			9 40	
1st December 1926				14 00			9 °8	
8th December 1926				13 86			9 17	
15th December 19 6	422	78 1°		13 7_	1 0		9 06	1 2
2_nd December, 19°6				13 58			8 9_	
29th December 1926			Moist powder	13 46		Dry powder	8 84	
5th January 19°7				13 32			8 73	
12th January 1927				13 19			8 6°	
19th January 19_7	4_°	6 5°		13 06			8 52	
°6th January 1927				1°93			8 42	
2nd February 19_7				12 80			8 32	
9th February, 19°7				12 67			8 2°	
16th February 1927	471	80·8°		12 54			8 12	
°3rd February 19°7				1° 42			8 0°	

BLEACHING POWDER IN AUTHOR'S EARTHENWARE JARS

TABLE III

Result expressed as percentage of Av Cl in the middle samples

Rains

Date	Average Monthly Absolute Humidity	Average Monthly Maximum Temperature Far	Physical Condition	Pure	Average fall per week	Physical Condition	Stabilized	Average fall per week
					Per cent			Per cent
30th June 1926				28 50			28 50	
7th July, 1926				27 90			27 70	
14th July 1926	974	90 1°		27 37			27 00	
21st July 1926				26 82			26 33	
28th July 1926				26 29			25 68	
4th August 1926				25 76			25 05	
11th August 1926				25 25	2 0		24 43	2 5
18th August 1926	982	88 0°		24 74			23 82	
25th August 1926				24 25			23 22	
1st September 1926			Dry powder	23 77		Dry powder	22 64	
8th September 1926				23 30			22 08	
15th September 1926	9[illegible]3	88 3°		22 84			21 53	
22nd September, 1926				22 38			21 00	
29th September 1926				21 93			20 48	
6th October, 1926				21 49			19 87	
13th October 1926				21 06			19 38	
20th October 1926	753	89 9°		20 64			18 90	
27th October 1926				20 23			18 43	
3rd November 1926				19 83			17 97	
10th November 1926	478	83 2°		19 44			17 5[illegible]	

Dry cold weather

Date	Average Monthly Absolute Hum dity	Average Monthly Max mum Temperature Far	Phys cal Condit on	Pure	Average fall per week	Phys cal Cond tion	Stabilized.	Average fall per week
					Per cent			Per cent
10th November 19 6				19 44			17 52	-
17th November 19 6	478	83 °		19 18			17 17	
°4th November 19°6				18 93			16 83	
1st December 19 6				18 68			16 50	
8th December 19°6				18 43			16 17	
15th December 19 6	4°2	78 6°		18 19			15 85	
22nd December 19°6				17 95	1 33		15 53	° 0
°9th December 19°6			Dry powder	17 71		Dry powder	15 °°	
5th January 19°7				17 47			14 9°	
12th January 19°7				17 24			14 6°	
19th January 19°7	4°	6 5°		17 01			14 33	
°°tl January 19 7				16 78			14·04	
°nd February 19°7				16 56			13 76	
9th February 19°7				16 34			13 49	
16th February 19°7				16 12			13 °°	
°3rd February 19°7	471	80·8°		15 91			1° 96	
° d March 19 7				15 70			1° 0	

Dry hot weather

Date	Average Monthly Absolute Humidity	Average Monthly Maximum Temperature Far	Physical Condition	Pure	Average fall per week	Physical Condition	Stabilized	Average fall per week
					Per cent			Per cent
2nd March 1927				15 70			12 70	
9th March 1927				15 43			12 41	
16th March 1927	550	89 8°		15 16			12 13	
23rd March 19?7				14 89			11 86	
30th March 19 7				14 63			11 60	
6th April 19_7				14 37			11 33	•
13th April 19 7				14 1?	1 75		11 08	2 ?5
?0th April, 19?7	788	103 7°	Dry powder	13 87		Dry powder	10·83	
27th April 1927				13 63			10 58	
4th May 1927				13 39			10 34	
11th May 1927				13 15			10·11	
18th May 19_7	893	99 1°		12 9_			9 88	
25th May 19?7				12 69			9 66	
1st June 1927				12 47			9 44	
8th June 1927	984	97 5°		12 25			9 2?	
15th June 19?7				12 04			9 01	

PERCHLORON

TABLE IV

Period of year	Date	Number of weeks	In Closed Iron Drum: Physical Condition	In Closed Iron Drum: Percentage of available Chlorine	In Closed Iron Drum: Average loss per week	In Open Kerosine Tin: Physical Condition	In Open Kerosine Tin: Percentage of available Chlorine	In Open Kerosine Tin: Average loss per week
					Per cent			Per cent
Dryhot weather	6th May 19?7	4 weeks	Dry powd r	67 4	0 13	Dry powder	6 4	0·45
	13th May 19 7			67 3			67 04	
	?4th May 19?7			67 ?			66 7	
	27th May 19?7			67 1			66 4	
	2nd June 19?7			67 0			6? 1	
Rains	9th June 19?7	?0 weeks	D y powder	66 7	0 6	Mo st powder	64 8	? 9
	17th June 19?7			65 8			6? 5	
	24th June 19?7			65 2			59 8	
	30th June 19?7			65 1			58 2	
	8th July 19?7			64 9			57 4	
	15th July 19 7			64 7			56 6	
	? n l July 19 7			64 4			5? 4	
	?9th July 19 7			63 8			54 4	
	5th August 19?7			63 ?			53 1	
	1?th August, 19 7			6 9			51 8	
	18th August, 19?7			6 8			50·5	
	?6th August 19?7			6? 6			48 5	
	?nd September 19 7			6? 4			47 1	
	9th September 19?7			61 9			45 4	
	16th September 19 7			61 4			43 8	
	23rd September 19 7			60 5			40 ?	
	12th October 19?7			59 9			34 8	
	?1st October 19?7			59 4			3 6	
	?8tl October 19?7			59·0			30·?	
	5th November 19?7			58 7			?9 ?	
	11th November 19?7			58 4			?8 ?	

APPENDIX II

Experiment on Bleaching Powder (Pure and 'Stabilized') for noting the difference of temperatures
(Started at 1 30 P M on 25th November, 1927)

	25 11 27	26 11 27		28 11 27		29 11 27		30 11 27		1 12 27		2 12 27		3 12 27
	2 P M	10 30 A M	4 P M	10 30 A M	4 P M	10 30 A M	4 P M	10 30 A M	4 P M	10 30 A M	4 P M	10 30 A M	4 P M	10 30 A M
Stabilized	34 0*	25 3	27 2	24 8	26 2	24 0	25 3	24 0	25 0	23 9	25 3	23 8	25 1	23 8
Pure	25 0	24 2	25 5	24 2	25 0	23 9	25 0	23 8	24 7	23 8	24 7	23 8	24 9	23 8

* Results are expressed in degrees in Centigrade Scale

DISCUSSION

Dr N L Banerjea (Bengal) I beg to differ from Dr Tomb regarding his contention that the percentage deterioration of bleaching powder alone was greater than that of bleaching powder mixed with lime Experiments have been done in the hygiene laboratory of the Calcutta School of Tropical Medicine where it was found that there was a certain optimum proportion of lime for bleaching powder which was more stable than the bleaching powder itself With regard to the stability of 'Perchloron' as compared with bleaching powder I agree with Dr Tomb The result of the work done in the School laboratory will be embodied in a paper to be published and Dr Tomb will then have the satisfaction of comparing my results with his I am of opinion that the quality of lime used by Dr Tomb had something to do with the difference in our respective results

Dr B C P Jansen (Netherlands East Indies) In Java a German preparation was imported under the name of Caporit which had the same good qualities Dr Tomb mentioned for Perchloron Further it had struck him very much that the stabilizing effects of quicklime on chloride of lime had been found to be nothing and he himself used NaOH for stabilizing NaOCl with very good results in his laboratory

Dr J W Tomb (Bihar and Orissa) replied The solution referred to by Dr Jansen was known in the British Pharmacopœia as Liq Sodæ Chlorinatæ It was well known and proved to be a very stable preparation With regard to the other speakers remarks the investigations described were carried out twice over the three seasons of the year According to the directions of the original paper of Aumonier and Elliott 20 per cent dry and recently burnt quicklime was used The first effect was to send up the temperature of the mixture 10° C for several hours above that of the bleaching powder with a marked fall of available chlorine of the stabilized mixture

OPIUM HABIT IN INDIA.

BY

LIEUT COL R N CHOPRA, M A , M D (Cantab), I M S ,

AND

KHEM SINGH GREWAL, M B B S (Lahore),

Department of Pharmacology Calcutta School of Tropical Medicine

Historical and General

THAT the oldest addiction drug in India is *Cannabis indica* is shown by the fact that reference has been made to it in the ancient Sanskrit books written before the Christian era The opium habit is of comparatively recent origin and it appears to have come gradually into being after the poppy was introduced into the country by the Mohammedans No reference has been made in the ancient books on Hindu medicine either to poppy or its products The medical writers of the period after the Mohammedan invasion only refer to the use of this drug in the treatment of disease and it is said that even the Sanskrit name of the drug is derived from the Persian word 'Afiun' The oldest work on Hindu medicine in which opium is mentioned is Rajanirghanta compiled about six hundred years ago and even in this book the reference made is very brief It is said to cure concurrent derangements of the three humours, increase sexual and muscular power and produce stupification of the brain Later Bhavaprakash, written 300 years ago by Bhava misra the distinguished physician of Benares, described its action and its uses more in detail From the testimony of historical records it is quite clear that the Arabs were responsible for the introduction of opium into India as into many of the other Eastern countries, and that its medical and other uses started from that time

Production of Opium in India and control exercised over it

The poppy could be grown in most parts of India and after its first advent into this country it was cultivated at first along the sea coast areas and later grown in the interior of the peninsula So extensively was it grown in the time of the Moghuls that opium became an important article of trade with China and other eastern countries At the time of the Emperor Akbar, its importance as a source of revenue was first appreciated and it was he who made a state monopoly of it The drug appears to have been largely used by the rich classes as a luxury under the Moghuls

After the fall of the Moghul Empire the monopoly passed to the East India Company and since then, though changes have been made in the method of control, in production, distribution, sale and possession of opium, the Government have held the sole control of the whole produce in British India Under the East India Company the general cultivation of poppy and production of opium were prohibited, these being restricted to three centres —(1) Patna or Bengal opium from poppy grown in Bihar and Bengal, (2) Malwa opium from Rajputana and Central India, and (3) Benares opium from the United Provinces Besides this, in the Punjab and also in the Simla hills poppy was grown to a small extent for local consumption.

According to the statistics collected by Watt in 1881, the total area under poppy cultivation in British India did not exceed 1,000,000 acres and that it had been stationary for the past 30 years The average yield per acre was about 15 to 20 pounds of opium and it was calculated that roughly not more than about 20,000,000 pounds of opium was produced A large part of it was meant for export, a comparatively small quantity being kept for consumption at home

The internal policy of the Government of India has been and is, one of non interference with the moderate use of raw opium, whether the object of the consumer be some real or supposed physical benefit, or merely the indulgence of the almost universal desire of human beings (particularly those whose occupations involve exposure, or severe bodily exertion), for a stimulant or a narcotic Excessive indulgence it is, and always has been, the desire of the Government to suppress The manufacture, possession, transport import, export and sale of opium are strictly controlled under an Opium Act of 1878 An individual can obtain opium only from a licensed retail vendor or a licensed druggist Each stage of the distribution down to the retail vendor is safeguarded by an elaborate system of transport passes, while the conditions designed to restrict abuse governing the licence of a retail vendor are most stringent He may not sell to any one person at one time more than the quantity of opium that an individual may lawfully possess, he may sell only for cash and only on premises for which he is licensed, he must not allow consumption on such premises, and he must keep correct daily accounts of his sales, which are open at all times to inspection by excise officers As regards exports, the Government of India, as a result of an agreement concluded with the Chinese Government, began in 1908 to diminish progressively the total amount of opium sold at Calcutta for export, and since 1913 they have resolutely maintained the prohibition of export of opium to China One of the provisions of the Hague Convention of 1912 that raw opium shall not be exported to countries that prohibit its import has always been strictly observed by the Government of India, and since 1915 it has also been their policy to enter into direct sale agreements with the Governments of the importing countries who are responsible (as signatories to the Hague Convention) for limiting imports to 'legitimate' requirements and for preventing export With effect from January 1923, the 'Import Certificate System' prescribed by the League of Nations has also been adopted In 1926, the Government of India launched upon a new export policy With effect from 7th April, 1926, the

public auctions at Calcutta have been discontinued, and from that date no opium can be exported to the Far East except under a direct agreement with the Government of the importing country Further, the Government decided to extinguish exports to the Far East in ten years that is no opium will be exported for purposes other than medical and scientific after 31st December, 1935

The result of all this is that the cultivation of the poppy and the production of opium has been cut down enormously as will be seen from the following table —

	Acres	Produce in lb
1881	536 282	7,800,521
1920	164 621	1,870,436
1921	116,055	1,179 977
1922	117,932	1,518 828
1927	52,279	885 641

Both the export and internal consumption of opium were decreased Thus exports fell from 69 708 chests in 1900 01 to 10 509 chests in 1919 20 and in later years has fallen still further (export chest = 140 lb)

It will be seen, therefore that the production of opium in parts of India under the British administration, which comprises an area of about two thirds of the whole country is strictly controlled and the cultivation of the poppy is being progressively reduced every year A little over one third of the country, however, comes under the control of Indian Princes and over the growth of poppy in these States the Government has no control While no opium produced within these territories can pass into British India or to any of the seaports, except under permit from the Government of India it is difficult for the Government of India to exercise any control over the production for the purpose of internal consumption The Government however, is doing all that lies in its power to bring these States into line with the policy adopted by the Imperial Government in accordance with the terms of the Hague Convention and a number of the Indian States has already agreed to similar restrictions being imposed in their territories A conference was held this year in Simla where all the representatives of different Indian States were present and it is hoped that further progress will be made in this direction

The Extent of Opium Addiction in India

As is well known opium is generally consumed in India in the form of a pill or it may be dissolved in water and drunk Of recent years another method of taking opium has come into vogue This consists in boiling the drug with tea and taking the infusion as tea This method though it looks innocent enough is dangerous from the point of view of the spread of the habit and we have come across instances where persons have unknowingly become addicts by partaking of this tea Poppy capsules from which opium is not extracted, were at one time largely used all over India and the custom of taking a decoction made from these capsules is not uncommon even now in some of the central districts of the Punjab Owing

to more strict control of poppy cultivation this custom is rapidly disappearing The smoking of opium with the exception of Assam is negligible in the country

national question The questions we have to consider in answering these criticisms are (i) Is opium addiction very common in India ? (ii) Is the habit spreading ? *(iii) Is the consumption of opium so great as to be a menace to the health and the* morality of the people in general ? Although no statistical data regarding the number of addicts are available a careful study of the question shows that though the opium habit may have been very prevalent in the 17th and 18th centuries it never could have been so widely spread and abused as it has been in other eastern countries The ravages which it is said to have produced in countries like China and elsewhere have no parallel so far as India is concerned

Sir William Robert in his minute in the Opium Commission Report says Taking India as a whole it may be said that a small minority even of the adult male popula*tion take opium habitually* *That was 32 years ago* We have *carefully gone* into the matter and have collected statistics from the excise records and from work in the field to gauge the extent of opium consumption in the country at the present time We have been forcibly struck by the fact that the opium habit is not nearly so common in India now as might be imagined from some of the recent publications by authors who have interested themselves in this question Although opium is still administered extensively to infants in many parts the habit is not widely disseminated among the adult population throughout India *Its incidence among* various peoples is very irregular and although there are certain areas and certain classes of population which are badly affected these form a *very small minority* Our investigations in the Punjab show that in the central districts of that province—Ferozpur Ludhiana and Ambala—which are populated chiefly by the Sikhs and where the consumption of opium recorded is one of the highest in the whole of India, with the exception perhaps of Assam and Calcutta the percentage of addicts is about 0 1 per cent of the total population In most of the other districts of that province the consumption of opium is up to the standard laid down by the League of Nations as being *necessary* for medical and *scientific purposes and the number* of addicts in these areas is not even 1 in 20 000 to 1 in 25 000

We have also gone into the question of doping of infants and children with opium Our detailed investigations at present are confined to the Punjab and we find that the custom is quite uncommon even in the central districts where the habit is very prevalent among the adults From a rough general survey of most parts of India we have made it appears that this custom chiefly prevails at present among the population of areas around large industrial centres *but on the whole* the practice is becoming less and less common in the rural areas and has entirely disappeared in many parts where it existed before Basing our opinion on our investigations in the Punjab again we have also been impressed with the fact that

opium habit among the aged is not nearly so prevalent now as it was two or three decades ago Although one does come across people who take small quantities—½ to 3 or 4 grains a day—when they have passed the age of 45 or 50 and when their vitality is on the wane *this is by no means a common practice in the* Punjab and in most other parts of India at the present time

We have therefore come to the conclusion that the opium habit in India is not very common and is certainly not spreading In fact our investigations in the field go to show that during the last fifteen years the addiction has considerably decreased This is also obvious from the fact that the quantity of excise opium issued for consumption in British India including Burma during 1925 26 was roughly 600 748 lb as compared with 855 721 lb in 1919 20 and 1 031 27 lb in 1911 12 The decrease is much more marked in the last few years than in the previous decade The factors which have been chiefly instrumental in reducing the consumption of opium in India are the decrease in its production and increase in its price In most provinces the retail price of opium has been increased to twice and in some provinces to three times of what it was in 1911 12 Some people have *advocated a further increase in price so as to make its use prohibitive from the* nature of its costliness As has been rightly pointed out this will only lead to increased smuggling from the Indian States Persia and Afghanistan and defeat its own object We have found that this is taking place even as the prices are at present and in spite of all the vigilance of the excise department opium is being smuggled into those areas where the price is higher than others

The Indian Addict

The next question is—what is the effect of the opium habit on the addict? This question can only be answered by patient work in the field and observations on a large number of addicts and during the last two years we have been able to study more than seven hundred addicts both in the towns and rural areas

From the data we have collected the Indian addicts to opium are divisible into *three main groups* —

Under the *first group* come all those persons who resort to the drug because they found it gave them relief from certain diseases or minor ailments from which they were suffering and as many as 30 to 40 per cent of the addicts in our series came under this category Though the State is doing all that is possible to increase the facilities for medical aid to the masses the majority of the population still resort to very primitive methods of treatment and that accounts for opium being used as a household remedy We have come across large areas, where though medical aid on western lines is available people will not take advantage of it through ignorance and prejudice *against it They go on using such drugs as opium* failing to realize the fact that they are merely palliative and have no curative value Most of these people start with a small dose and stick to it The euphoric action of the drug has no attraction for them relief of their ailment being the paramount consideration A number of this group fall victims to the euphoric action of the drug also and

increase the dose in the same way as an addict who has contracted the habit fo its stimulant action

It is worthy of note here that in our series most of the diseases for whicl opium was used (and the habit was formed) were of a minor character, th commonest being pain of a neuralgic type, joint pains and alimentary anc respiratory diseases The drug was never started on medical advice, but ir most cases on the advice of a friend (who possibly was an addict himself) or oı the sufferer's own initiative In our series of cases we did not find a singl case of severe or incurable disease for which opium was taken either on medica or lay advice

The *second group* comprising about 10 to 20 per cent includes those people whc have been working under strain and stress of life unbearable to them and take th drug to forget their worries and anxieties These may be the tillers of the soil o may follow other vocations in life The social and economic conditions prevailin in the country often throw a lot of strain especially on the elder or earning member of the family People often have to work hard right up to a very ripe old age owin to the loss of the younger earning members of the family They start opium witl the idea of staving off the effect of old age so as to enable them to carry on thei work but really it does nothing more than to depress their sensitivity to thei surroundings, and does harm in the long run The dosage taken by these twc groups is as a rule not large, or progressive and in the majority of cases it keep well under 10 grains a day

The *third group* of addicts or the euphoric type consists of those persons whc get to taking opium for the purpose of self gratification and comprise about 3(to 40 per cent of the total addicts These people are generally well off and star taking the drug for its stimulant action sexual pleasure or for the comfort which i affords them In this class of cases the psycho neurotic factor plays an importan part and this type forms quite a large portion of the younger addicts in our series The addicts are generally between the ages of 20 and 35 and this class of addict i on the increase both in cities and rural areas while the others show a decrease Th old restraining influence of the elders on younger men even in the village appear to be decreasing and addiction to opium and alcohol is increasing among them Under this group are also included a large criminal class who often start takin opium under the impression that it fortifies them and enables them to bear th physical and mental strain connected with their nefarious work They ofter increase the dose and the euphoric factor becomes prominent in many of them and they get addicted to other drugs such as *Cannabis indica* cocaine etc Ou investigations in the jails show that a large percentage of the population are addicted to opium

In all these groups association with other addicts plays a very important part in starting the habit

Dosage —As regards the quantity of opium consumed daily by different groups of addicts the dose taken by the first two groups as a rule is small A small

number about 15 to 20 per cent of total addicts take less than 5 grains a day but most of them do not exceed 10 to 12 grains a day Although not infrequently one meets individuals taking over 15 to 20 grains a day in the first two groups it is in the third group that one must look for addicts taking large doses such as 20 to 30 grains a day or over The latter form about 15 to 20 per cent of the total addict population but even in this class it is rare to find the so called opium fiend During the last two years we have not come across a single case where opium was taken in doses corresponding to those of morphine said to be taken by the morphine addicts in America One reason of this is that owing to the high price of opium even those persons who took very large doses have had to decrease them It will be seen therefore that about 15 to 20 per cent take small doses below 5 grains a day 60 to 70 per cent take doses ranging between 10 to 20 grains per day and 15 to 20 per cent take doses over 20 grains a day The average dose in this series of 700 cases worked out to be 17 63 grains per day

Effects on the Health

It is not possible for us to deal fully with the effects the opium habit produces on the health of the individual who is addicted to it We are going to deal with these in a separate paper It may be stated however that the addicts who generally show no outward signs of the habit either physical or mental and who consider the habit is not doing them any harm (often think it is doing them good) are those who take small doses not exceeding 5 grains a day Most of the other addicts tell you that the habit is doing them harm They say that they have not the same energy and vigour as they had before, they are unable to do hard physical work for any length of time and they are incapable of mentally concentrating their attention to do any highly intellectual work Our own experience bears out these facts and we find that generally those habitués who take more than 5 grains a day become both physically and mentally dull Their output of work is not equal to that of non eaters of opium From the general appearance it is often not possible to tell an addict who is taking less than 5 grains of opium per day Most of those taking larger doses can be generally spotted from their emaciated appearance vacant and sleepy eyes and a peculiar sallow colour of their face A cursory examination of these individuals gives the impression that they are not only suffering from the narcotic effects of the drug but also from some form of intestinal toxæmia Small doses would therefore appear to produce apparently little effect but with larger doses there are obvious signs of deterioration of the general health of the individual

Summary and Conclusions

From our work in the field for the past two years we feel justified in drawing the following conclusions regarding the opium habit in this country —

(1) The opium habit is not nearly so common in India at the present time as might be imagined from some of recent publications on the subject The habit

is not widely disseminated among the population its incidence among the people is very irregular and although there are admittedly certain areas and certain classes of population which are badly affected these consist of a very small minority

(2) The habit is not spreading in fact during the last fifteen years it has considerably decreased both among the infant and the adult population.

(3) The factors which have been chiefly responsible for reducing the consumption of opium in this country are (*a*) decrease in its production and (*b*) increase in the price This last measure cannot be pushed any further at present because of the danger of smuggling but for localities where the incidence of addicts is very high other means should be devised

(4) About 30 to 40 per cent of the addicts start the habit by using it as a household remedy for relief of ailments 10 to 20 per cent take it to relieve strain and stress of life and in the hope staving off the effects of old age and 30 to 40 per cent for its euphoric effects This last class comprises chiefly young people between the age of 20 to 30 years

(5) The dose taken by the first two groups is small and as a rule not progressive, 15 to 20 per cent take under 5 grains a day but the majority under 15 grains a day The last group take large and increasing doses The average dose in our series of 600 cases worked out at 17 65 grains per day

(6) Those who take under 5 grains a day do not show any apparent physical or mental changes but those taking larger doses generally show a slowing of their physical and mental faculties and symptoms of chronic toxæmia

REFERENCE.

CHOPRA R N and GREWAL, KHEM SINGH (1927) Opium Habit in India. *Ind Jour Med Res* Vol. XV No. 1 July

number about 15 to 20 per cent of total addicts take less than 5 grains a day but most of them do not exceed 10 to 12 grains a day Although not infrequently one meets individuals taking over 15 to 20 grains a day in the first two groups it is in the third group that one must look for addicts taking large doses such as 20 to 30 grains a day or over The latter form about 15 to 20 per cent of the total addict population but even in this class it is rare to find the so called 'opium fiend' During the last two years *we have not come across a single case where* opium was taken in doses corresponding to those of morphine said to be taken by the morphine addicts in America One reason of this is that owing to the high price of opium even those persons who took very large doses have had to decrease them It will be seen, therefore that about 15 to 20 per cent take small doses below 5 grains a day 60 to 70 per cent take doses ranging between 10 to 20 grains per day and 15 to 20 per cent take doses over 20 grains a day The average *dose in this series of 700 cases worked out to be 17 63 grains per day*

Effects on the Health

It is not possible for us to deal fully with the effects the opium habit produces on the health of the individual who is addicted to it We are going to deal with these in a separate paper It may be stated however that the addicts who generally show no outward signs of the habit either physical or mental and who *consider the habit is not doing them any harm (often think it is doing them good)* are those who take small doses not exceeding 5 grains a day Most of the other addicts tell you that the habit is doing them harm They say that they have not the same energy and vigour as they had before they are unable to do hard physical work for any length of time and they are incapable of mentally concentrating their attention to do any highly intellectual work Our own experience bears out these facts and we find that generally those habitués who *take more than 5 grains a day become both physically and mentally* dull Their output of work is not equal to that of non eaters of opium From the general appearance it is often not possible to tell an addict who is taking less than 5 grains of opium per day Most of those taking larger doses can be generally spotted from their emaciated appearance vacant and sleepy eyes and a peculiar sallow colour of their face A cursory examination of these individuals gives the impression *that they are not only suffering from the narcotic effects of the drug but also* from some form of intestinal toxæmia Small doses would therefore appear to produce apparently little effect but with larger doses there are obvious signs of deterioration of the general health of the individual

SUMMARY AND CONCLUSIONS

From our work in the field for the past two years we feel justified in drawing the following conclusions regarding the opium habit in this country —

(1) The opium habit is not nearly so common in India, at the present time as might be imagined from some of recent publications on the subject The habit

is not widely disseminated among the population, its incidence among the people is very irregular, and although there are admittedly certain areas and certain classes of population which are badly affected, these consist of a very small minority

(2) The habit is not spreading, in fact, during the last fifteen years it has considerably decreased both among the infant and the adult population

(3) The factors which have been chiefly responsible for reducing the consumption of opium in this country are (*a*) decrease in its production, and (*b*) increase in the price This last measure cannot be pushed any further at present because of the danger of smuggling but for localities where the incidence of addicts is very high, other means should be devised

(4) About 30 to 40 per cent of the addicts start the habit by using it as a household remedy for relief of ailments 10 to 20 per cent take it to relieve strain and stress of life and in the hope staving off the effects of old age, and 30 to 40 per cent for its euphoric effects This last class comprises chiefly young people between the age of 20 to 30 years

(5) The dose taken by the first two groups is small and as a rule not progressive, 15 to 20 per cent take under 5 grains a day but the majority under 15 grains a day The last group take large and increasing doses The average dose in our series of 600 cases worked out at 17 65 grains per day

(6) Those who take under 5 grains a day do not show any apparent physical or mental changes but those taking larger doses generally show a slowing of their physical and mental faculties and symptoms of chronic toxæmia

REFERENCE

CHOPRA, R N and GREWAL, KHEM SINGH (1927) Opium Habit in India *Ind Jour Med Res*, Vol. XV No 1 July

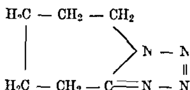
STUDY ON THE NEW SYNTHETIC ANALEPTIC CARDIAZOL

BY

A KESSLER, M D,

Professor of Pharmacology Tung Chu University, Shanghai

THE new analeptic *Cardiazol*, the pharmacology of which has already been studied by Hildrebrandt and his co workers as well as by other authorities, has a structural formula as shown below —



It is a white crystalline powder, having a slight bitter taste but no smell Its melting point lies between 56° to 58° C It has this advantage over camphor, which is to be substituted by the former in both its pharmacological and clinical aspects and even surpassed by it and over the other up to date substitute products that it is soluble in water in its true sense

The watery solution is neutral and consequently fit for subcutaneous application without producing any irritation Moreover, it is not affected by any chemicals This property is essential for its stability and undisassociation in the watery solution when sterilized

I have particular reasons for bringing before you the results of my study and experience concerning this new analeptic

I got the best results in the numerous cases of serious opium poisoning as well as in other serious cases of intoxication accompanied by paralysis of the circulatory and respiratory system

Further, we could convince ourselves of the beneficial and exciting action of this drug when given before and after the operation in case of accidents during operations Moreover we obtained always good results in cases of pneumonia, typhoid and dysentery, which show involvement of circulatory and respiratory systems The latest and partly published experiences about the effect on the circulation and in beri beri malaria dysentery Chagas' disease yellow fever and ankylostomiasis verify the pharmacological findings and conclusions

For the above mentioned grounds I was lead by the following experiments 'To damage the experimental object to a great extent or if possible to bring about a complete extinction of its normal function in order to study the highest efficacy index of cardiazol' This I held to be of special importance particularly when the unfavourable climatic conditions are considered with which surgeons as well as internists are confronted while treating their patients during the hot seasons of the year

The experiments were carried out on isolated frog hearts on rabbits and cats by means of a Straub s cannula Unfortunately the experiments on the isolated hearts of warm blooded animals must be omitted owing to some defect in the apparatus

The experiments on frog heart were carried out during the cold season of the year Ringer Locke solution with 0 1 per cent of grape sugar served as the nutritive fluid The solution was always freshly prepared and pure oxygen was allowed to pass through it for two hours The same volume of liquid was used whenever the liquid was renewed

At first the limits between the absolute irreversible poisoning action and the harmless but still sufficiently efficacious dose of cardiazol were studied

We observed severe poisoning effect by the concentrations of 1 25 and 1 50 but both of these concentrations behaved in respect to their end effects in quite an opposite manner A solution of 1 100 caused a distinct but temporary damage which was manifested by a stronger systolic and diastolic regular heart function

In case of a concentration of 1 25 the ventricle comes to a standstill with a fairly strong contraction after 20 seconds while the auricles of the heart are strongly dilated but show a regular but weak contraction

After about 90 seconds the auricles exert only a weak jerk on the sinus knot while the ventricle is by this time extended to its maximum After further 40 seconds the ventricle and the auricles finally come to standstill After 140 seconds there is a temporary slight contraction This is followed by dilatation After further 150 seconds the contraction of the ventricle begins with a gradual jerk followed by a strong contraction of the auricles The heart remains finally in this condition which is not changed by washing it with Ringer Locke solution or by the pressure of a column of liquid

The concentration of 1 50 produces again relaxation of the ventricle and the auricles which is preceded by diminution of the ventricle diastoles but at a greater interval of time Shortly after we observe both the ventricle and the auricles in the position of highest dilatation the ventricle completely at rest and the auricles making only feeble rolling movements After about nearly six minutes the ventricle begins all of a sudden to answer to every motion of the auricles which still remain in a dilated condition with a contraction at normal intervals Afterwards the dilated auricles seldom show

any movement and finally both auricles and ventricles come to a standstill This condition remains unchanged provided the heart is not washed with Ringer Locke solution If washed out the normal functions of heart return an increase of the ventricle diastoles and systoles can be observed a symptom which could be noticed in all cardiazol studies on normal and damaged hearts and is similar to digitalis substance in its action

At the concentration of 1 100 the appearance of extra systole of the ventricle and temporary damage of the diastole are the signs of the poisoning action A normal heart function which however differs from the original heart function gradually develops

At the concentration of 1 200 the damaging action of cardiazol can no longer be observed on the contrary we observe a favourable effect on the chronotropic and inotropic property of the isolated frog heart It is possible to ascertain an undisputed direct and useful action of cardiazol on the normal heart contrary to camphor

In order to study the effect of cardiazol on an isolated frog heart damaged by chloroform a saturated solution of chloroform was taken It is prepared by shaking chloroform with a Ringer Locke solution After four or five hours the supernatant poured off liquid is called saturated chloroform solution which is to be diluted in the proportions 1 5 and 1 2 in order to *study the influence of cardiazol on the most extreme cases of heart poisoning* by chloroform

The dilution of 1 5 produces the above mentioned contraction of the ventricle partially damaged which similarly dilates with a slight systole accompanied by an immediate dilatation of the auricles

A cardiazol solution of 1 1000 dilution shows a ready effect In about three minutes the original power of the heart has been completely restored and strengthened

The heart is damaged to the extreme with a solution of chloroform 1 1 The application of this solution dilates the auricles and ventricles immediately The heart comes to a standstill The cardiazol solution 1 1000 shows a positive but a time consuming reaction An instantaneous reaction and re establishment of systoles and diastoles at regular intervals are obtained by a cardiazol solution of 1 500

As a finishing touch to the chloroform cardiazol experiments the influence of a cardiazol concentration of 1 1000 on the damaging action of a strong chloroform dose (1+1) was further studied when the total quantity of cardiazol solution (1 1000) was simultaneously added to the chloroform The cardiazol solution in this case had a very favourable influence on the course of the very serious poisoning effect arising from such a strong dose of chloroform There was only a slight and temporary impairment of the heart s power in place of the usual paralysis

Preliminary experiments were then undertaken to estimate the smallest dose of cardiazol which reacts on rabbits producing spasm when intravenously injected Five milligrams and 7 5 mg of cardiazol per kilo body weight of the animal have no effect at all Intravenous injection of 10 mg per kilo reacts immediately as a tonic produces clonic spasm accompanied by a biting tendency

Spasm lasts for a few seconds the biting tendency for two minutes After ten minutes the animal returns to its normal condition Intravenous injection of 15 mg per kilo causes a stronger poisoning A tonic clonic spasm lasting for 20 seconds is followed by an epileptic form of spasm with biting tendency for two *minutes Three minutes later the animal tries to stand up* Double the quantity of cardiazol 30 mg per kilo injures the animal more seriously It springs up in the air after the injection and remains for 30 seconds under the influence of a tonic clonic spasm followed by long clonic and epileptic spasms with a biting tendency lasting for an hour *After this interval the animal gets up again* voluntarily but seems to be tired for two hours

The above mentioned experiment on young rabbits can be made every alternate day on the same animal without observing any change in the normal condition of the animal after the subsidence of the poisoning effect The minimum fatal dose of cardiazol amounts to 45 mg per kilo of the body weight The jerks of the dilated ventricle at regular periods can be observed for eight minutes if the animal is *dissected just after its expiration The gastric peristalsis is normal blood* coloured black Death occurs from suffocation

The further investigation of the action of cardiazol on the circulatory as well as the respiratory system respiratory and blood pressures were at first separately and then simultaneously determined Respiration had been controlled in the following way Oxygen from a bomb was first allowed to pass through a glass bell dipped into a glass vessel containing water and then introduced into the trachea of the animal A washing bottle was placed between them by means of a two way system which was to be filled with chloroform if necessary for the later experiments with chloroform on cats The amount of oxygen introduced could at any moment be estimated by observing the positions of the water surfaces inside and outside the glass bell A current of chloroform could be produced by allowing the oxygen current to pass more rapidly Respiratory pressure was registered by the Marey Drum and the blood pressure by the double necked mercury manometer according to Ludwig

An animal weighing 2 kilos and hypnotized with urethan was injected with 5 mg of cardiazol per kilo repeated three times at the intervals of 60 seconds In this case no particular change except a temporary acceleration of no importance could be observed in the respiratory system

A very feeble reaction on the blood pressure of the rabbit hypnotized by urethan could be observed in the case of a similar concentration of the cardiazol Every new dose increases the blood pressure

Further, the course of the reaction on the blo system, following a dosage of three times the quantity In this case a much more positive reaction on the b the respiratory system, could be observed. After blood-pressure and the respiration, spasmodically acc blood-pressure, returned to its normal condition. The r than it was before the administration of cardiazol.

The influence of cardiazol on the respiratory syste by chloroform was as follows :—

After an intravenous injection of 5 mg. per of a cat, which had been brought to a standstill, beg sudden This dosage, in normal conditions, had no respiratory system. The condition did not last, so f concerned, for a long time It produced only a respiration

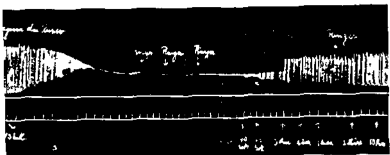
A rabbit hypnotized with urethan received 10 mg. This animal received further 5 mg. per kilo weight of was carried out to test the effect of cardiazol upon th dose proved insufficient to produce a rapid effect but cardiazol had an immediate effect upon the hypnotize

An antagonistic interaction of cardiazol and pant this experiment For the sake of completeness the e the subcutaneous administration of cardiazol. The noticed until a subcutaneous dose of 50 mg. per kilo dose, it will be noticed, when administered intraven the subcutaneous experiment the symptoms of spasm minutes These lasted for four minutes after which only to fall in another spasm after three minutes. The four minutes The condition of exhaustion lasted one

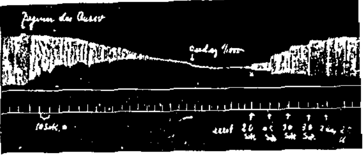
The course of the experiment becomes much clea diazol is registered by respiration-record. In this way t before the general appearance of the animal shows an to say, within 15 minutes when 20 mg. are injected sign on the part of the respiration of the effect of the c tion becomes deeper and, for a short time, quicker. T irritation is concerned, lasts a very short time and t respiration

In order to answer the question, *how long* the effe jection lasted, an over-poisoning dose, of 1 mg. of dico

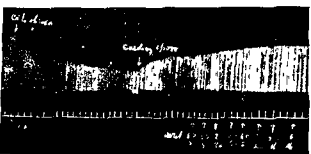
PLATE XXI.



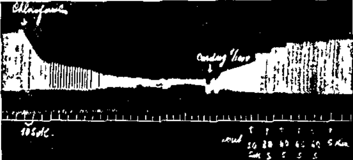
[Cu]rve 1 —Frog's heart isolated by the Straub cannula Action of Ringer Locke solution [on the] physiologically damaged heart.



Curve 2 —Frog's heart isolated by the Straub cannula. Action of cardiazol solution, 1 1000, on the physiologically damaged heart.

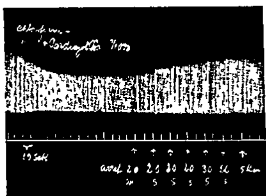


Curve 3 —Frog's heart isolated by the Straub cannula. Action of cardiazol solution, 1 · 1000, on the heart damaged by a chloroform solution; dilution, 1: 10.

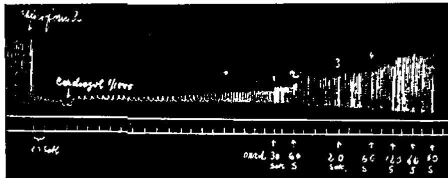


Curve 4 —Frog's heart isolated by the Straub cannula. Action of [cardiazol solution, 1 · 1000 ...]

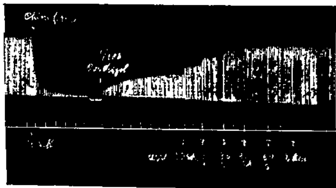
Plate XXII.



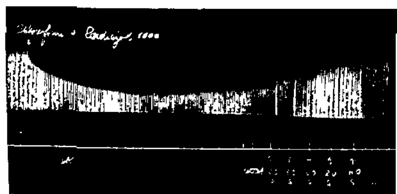
Curve 5.—Frog's heart isolated by the Straub cannula. Action of cardiazol solution, 1 · 1000, given simultaneously with chloroform solution, dilution, 1 5.



Curve 6.—Frog's heart isolated by the Straub cannula Action of cardiazol solution, 1 : 1000, on the heart damaged with chloroform solution, dilution, 1+1.

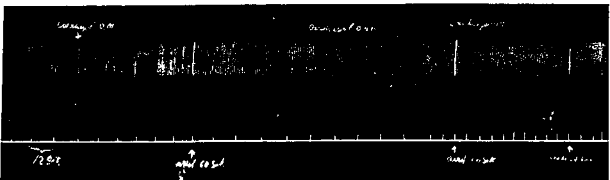


Curve 7.—Frog's heart isolated by the Straub cannula. Action of cardiazol solution, 1 : 500, on the heart damaged with chloroform solution; dilution, 1+1.

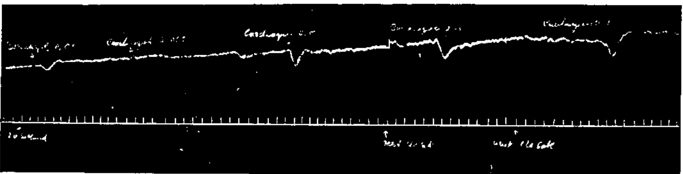


Curve 8.—Frog's heart isolated by the Straub cannula. Action of cardiazol solution, 1 : 1000, given simultaneously with chloroform solution; dilution, 1+1.

Plate XXIII.

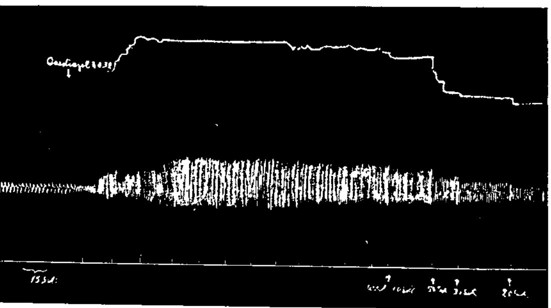


Curve 9 —Rabbits of 2 kilos weight Effect of 10 mg cardiazol on the respiration Injection repeated twice without any particular change in the respiration



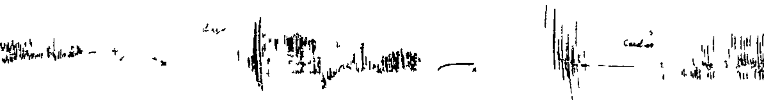
Curve 10 —Rabbits of 2 kilos weight Effect of 10 mg of cardiazol on the blood pressure. Blood pressure estimated by means of a simple \ shaped mercury manometer after Ludwig The first injection of 5 mg followed by three of 10 mg At +, restlessness of the animal.

Plate XXIV.



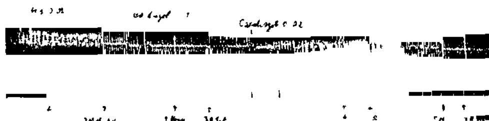
Curve 11.—Rabbit of 1·6 kilos weight. Action of 32 mg. cardiazol on the blood-pressure and respiration.

PLATE XXV

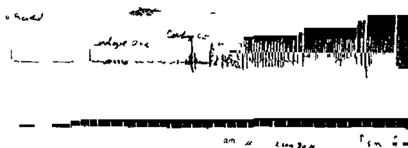


Curve 1°.—Cat of 3 kilos weight Action of 15 mg cardiazol on the respiration paralysed by chloroform
S d Atm m chl =Saturation of the respiration with chloroform
× =Chloroform stopped
× × =Administration of pure oxygen—marker removed from the curve for ten seconds.

PLATE XXVI.



Curve 13.—Rabbit of 2 kilos weight. Effect of 10 mg., then 20 mg., of cardiazol on the respiration damaged by 20 mg. pantopon.

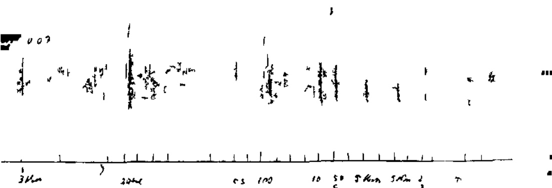


Curve 14.—Rabbit of 2 kilos weight. Action of 20 mg., followed by 10 mg., of cardiazol on the respiration paralysed by 6 mg. of dicodid.

PLATE XXVII



Curve 15 Rabbit of 1 75 kilos weight Effect of a subcutaneous inject on of 35 mg cardiazol on the respiration

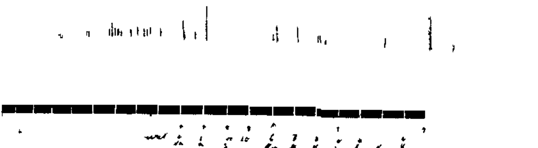


…e 16 —Rabbit of 1 75 kilos weight Effect of a subcutaneous injection of 70 mg of card azol on the respiration

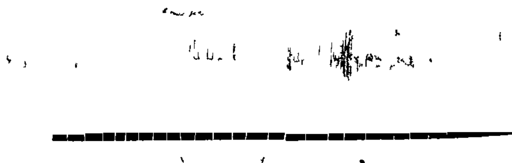
Curve 17.—Continuation of Curve 16

Curve 18.—Rabbit of 1.7 kilos weight. Effect of a subcutaneous injection of 70 mg., given two hours previously, on the ... reaction shown in Curve 19

PLATE XXIX



Curve 19 —Rabbit of 1 25 kilos weight Effect of 1 25 mg d codid on the respiration



Curve 20 —Rabbit of 1 6 kilos weight Action of 1 6 mg of d cod d followed by a subcutaneous injection of mg of cardiazol on the resp ration

Summary of Pharmacological Properties

(1) The absolute fatal dosage of cardiazol lies very far from that therapeutically effective one this property is a very favourable one cardiazol

(2) A reaction similar to digitalis can be established, according to Straub frog hearts

(3) The action of cardiazol on hearts highly damaged by chloroform is reliabl and instantaneous

(4) Simultaneous addition of cardiazol to chloroform diminishes or prevents the damaging action of the latter on the heart

(5) The spasmodic action caused by a large dose of cardiazol on warm blooded animals is similar to that of camphor

(6) Cardiazol is a strong dependable stimulant for the central respiratory and circulatory system

(7) Experiments on exciting the respiratory system and increasing the blood pressure of a decapitated cat give no favourable results

(8) Intravenous administration of doses which cause no spasm have no exciting effect on the normal respiratory system but slightly increase the blood pressure of rabbits hypnotized by urethan Repetition of such strong doses shows no cumulative effect

(9) Doses causing no spasmodic effect already produce an instantaneous action on the respiratory centre damaged by hypnotics

(10) The action of subcutaneous injections with cardiazol solution distinguishes itself from that of intravenous injections by the following facts —

It is slower in its reaction it is proportionally less toxic and its duration is longer

Subcutaneous injections are easily borne without the tissues being damaged thereby

The hypnotics of the morphin group and cardiazol are antagonists the simultaneous action of both produces a favourable effect on the respiratory system.

On the foundation of my pharmacological results and experience gained from the experiments made on the clinical materials I would like to mention that in ery serious cases the cardiazol dosage for subcutaneous injections should be ncreased by double and if necessary the normal dose of 0 1 grm should be re ated during the first three hours

In spite of the pharmacological results both subcutaneous and intravenous ections with cardiazol require the same time for their effects upon human ngs

From the results of recent and thorough experimental investigations cardiazol also been recommended to be administered *per os*

DISCUSSION

Lieut Col R N Chopra I M S (Bengal) Said he was very grateful to Dr Kessler for his interesting paper on Cardiazol He himself had no personal clinical experience of the drug and would like to ask Dr Kessler if he had tried it in cases other than opium poisoning

Dr A Kessler (China) replied Cardiazol was used very much by him and the other physicians on his advice not only in grave cases of opium poisoning with great success but also in cases of amœbic dysentery, pneumonia typhoid fever and other infectious diseases where the heart and circulation were badly affected In cases of pneumonia even better results were obtained on the respiration by the addition of dicodid or other bodies of the morphine group to cardiazol

SOME NOTES ON THE STUDY OF CHINESE DRUGS

BY

SEIKO KUBOTA

Department of Pharmacology Manchuria Medical College Japanese Concession Mukden China

IT may be said that there are two tendencies in the study of pharmacology at present the one endeavouring to get more effective new synthetic compounds which are derived from pure chemical substances the other striving to isolate some active principle from natural sources which may or may not be already known as a drug The former prevails chiefly in the West and the latter in the Orient

In my laboratory in the Manchuria Medical College several Chinese drugs have been the subject of our study and I am glad to have the opportunity to read a paper in this meeting on some notes on the study of Chinese drugs with the hope that this may help in some degree to encourage the study of these drugs in the Orient

I shall not go here into a detailed report on each drug which I have studied but I only wish to give some notes on what I have experienced in the course of studying as a general guide for those who are interested in studying the pharmacology of Chinese drugs

(1) *Review of Literature on the Subject*

There are many thousands of Chinese drugs which have a history of several thousand years and most of them are taken from the vegetable kingdom

On entering upon the investigation of Chinese drugs what perplexes us most is that there is some confusion between the names of drugs and the drugs themselves On the one hand one drug generally has several names sometimes ten or more As an example let me take Huang ching (*Polygonum giganteum*) it has as many as sixteen names which are as follows —

HUANG CHING

(*Polygonum giganteum*, Dietr var Thunbergii, Maxim)

Other names		
	黄芝	Huang shih
	戊己芝	Mou szu chih
	菟竹	T u chu
	鹿竹	Lu chu
	仙人餘糧	Hsien jen yu liang
	救窮草	Chiu ch'iung t'sao
	米餔	Mi pu
	野生薑	Yeh sheng chiang
	重樓	Chung lou
	雞格	Chi ke
	龍銜	Lung shieng
	垂珠	Chui chu
	葳蕤	Wei jui
	白及	Pai chi
	苟格	Kou kê
	馬箭	Ma tsien

On the other hand there are many evidences that the same name is sometimes applied to several different drugs, for instance, the name Hu su is used for K'uan-tung hua (*Tussilago farfara, L*) as well as for Sha shen (*Adenophora verticillata*, Fish) and for Teng hsing ts'ao (*Iuncus effusus, L*) also A number of such examples can be found in the paragraph of Yo ming tung 1 in Pen t'sao Kang Mu, a famous Chinese materia medica

On reading Chinese medical books in which many therapeutic experiences of drugs are written, we find very often some statements incomprehensible if we consider them according to the scientific knowledge of the present time But the study of this Chinese literature is always very useful not only that we may know the clinical and histological references but also to get some idea of the pharmacological action of the drugs.

(2) *Materials for Study*

Materials for study which we buy from a local druggist are sometimes not genuine and the commercial preparations are often contaminated with some impurity which is used for pharmaceutical manipulation in various ways, those are not suitable material for our study

To differentiate Chinese drugs needs some special knowledge and experience, the ordinary druggist of the present time, unless he is a good, experienced one, does not know much about it and makes mistakes easily Especially is this the case with drugs which are not in use in every-day treatment

Some years ago I obtained a drug under the name of Pan pien lien from a local drug merchant but to my disappointment I found it after several months of experiment not to be genuine so I had spent time and expense in vain The same bad experience was repeated with Hsia ku t'sao which was bought by me as a Lobelia plant from a local druggist

For each differentiation of Chinese drugs it is very important to know the original plant from which the drug is taken Though many efforts have been made by many investigators to find out the original plant for each drug, the identification is still incomplete Moreover, there is another inconvenience, namely, that several different plants may be called under one name in different localities, and it is no easy task to get these points clear If circumstances permit, it is best to cultivate the original plant and to collect the part desired as material for its chemical and pharmacological study.

(3) *Pharmacological Action of Chinese Drugs.*

For the isolation of the active principle from the Chinese drugs, heretofore, the organic substance was chiefly aimed at and discussed but the inorganic salts of the drug plant were not much considered In the experiments recently made, it was proved that many drug plants contain a considerable amount of inorganic salts which may display some action in clinical use.

Some years ago I once took as a subject of my study Hsia ku t'sao (*Brunnella vulgaris*) which was collected from several parts of China and on examination chemically and pharmacologically found a great amount of inorganic salts, but not any special organic active constituents, and most of the salts consisted of potassium salts The amount of extractive inorganic salts from the plant was calculated to be not less than 3 5 per cent of its weight, of which 68 per cent was

potassium chloride, 23 per cent potassium sulphate and for the remainder iron and sodium salts were found by analysis

Though the action of these inorganic salts is not so remarkable in nature as the specific organic substance which may be found in some other plants, it should be taken into consideration as well as the organic substance, in a discussion of Chinese drugs

Chinese drugs are used more often in a combination of several together rather than singly so that in discussion of their action we have to consider the combined action of the drugs used not only the action of each active principle, for we have many pharmacological evidences that, when two or more active principles are in combination the action of the one is often influenced by the other, causing an increase of the anticipated action and an elimination of some side action The former is known as the phenomenon of synergismus and the latter enables us to use the drugs more advantageously

In Chinese prescriptions we generally see many drugs written down, some times ten or fifteen or more, and it is believed in Chinese medicine that the action of Chinese drugs is manifested most remarkably when many are mixed together they say that the experience of many thousand years testifies to this

Those are the points which I wish to mention on this occasion under the title of ' Some Notes on the Study of Chinese Drugs '

DISCUSSION

Lieut Col R N Chopra, I M S (Bengal) Was very much interested in Dr Kubota s paper as he was working on the same lines in India He was interested to hear of the mixing of a number of drugs in Chinese prescriptions The same thing held good in India This was caused by the ignorance of the real action of the drugs The more the properties were studied the shorter would the prescriptions become When he told some of the practitioners of the indigenous system that a particular drug was useless they asked him if he had tried it with ten or more drugs ! There was a tendency to give what were called ' shot gun ' prescriptions

ON THE PHYSIOLOGICAL ACTION OF ANIONS

BY

N ONODERA, M D
E NISHIO
M YOSHIKI

AND

K YUKAWA

Kyushu Imperial University Fukuoka, Japan

If a concentrated solution (20—25 per cent) of sulphate of magnesia is directly imported into the duodenum an attack of diarrhœa will seldom ensue if however the same quantity of the same medicament is administered *per os* an attack of diarrhœa is bound to follow Facing these facts I supposed that when the medicament comes into contact with the mucous membrane of the stomach it produces something which accelerates peristalsis of the alimentary tract According to textbooks of pharmacology, the diarrhœa through sulphate of magnesia given internally is caused just by the failure of the sulphate ion to be absorbed from the intestinal canal Dr *Stransky really insists that the fact that Karlsbader Salz* causes an increase of bile flow from the gall bladder, depends upon the action of the absorbed sulphate ion on the gall bladder

Dr Nishina, of our laboratory causing an excretion of bile from the gall bladder by injecting into the vein 100 c cs of 2 per cent magnesium sulfricum solution supposed that the contact of this medicament with the mucous membrane of the stomach might produce some substance to cause contraction of the stomach the intestines and the gall bladder

Experiments were carried out following this supposition, Dr Nishina of our laboratory, employing the method of Dr Cannon Dr Carlson and Dr Kanegae who studied the movement of the stomach and the intestine by the means of an air balloon introduced in the intestinal canal injected into the vein several kinds of extract with the concentrated solution of sulphate of magnesia from several parts of the stomach, the intestines, and some other organs, thereby arriving at an interesting conclusion

Dr Nishina has found that there is some substance in the mucous membrane of the stomach of the rabbit, dog, pig and cow, which serves to heighten the tension

of the stomach wall, and to increase the peristaltic movement This substance does not however exist in the mucous membrane of the oral cavity, œsophagus the duodenum the intestines, nor outside the mucous membrane, nor in the liver and pancreas but is contained in a small quantity in the spleen only, if the solution of sulphate of magnesia is made slightly alkaline, no effective substance is extracted

When *sulphuric acid, hydrochloric acid, lactic acid, phosphoric acid, alcohol,* or saline solution as extracting fluids were employed, sulphuric acid (0 28—0 4 per cent) alone was effective This matter has been investigated by experiments again and again being enlarged upon by Dr Yukawa's study and it has been found that this substance acts upon the gall bladder, the small and the large intestines in the same manner as upon the stomach The extraction of the effective substance from the mucous membrane of the stomach was carried out with success either *when the stomach was empty, or during the process of digestion The active* substance passes through the dialyzing membrane and is not destroyed by half an hour's boiling nor has given any cholin reaction In these respects this active substance differs from Zulzer's peristaltic hormone, which was said to have been extracted from the mucous membrane of the stomach and in larger amount from the spleen with alcohol

From the above mentioned facts, one must reckon the evacuating action of *sulphate of magnesia upon the absorption of some substance,* which *heightens* the tension as well quickens the peristaltic movement of the stomach and intestines, and this substance is formed by the joint action of the sulphate ion, and some substance in the mucous membrane of the stomach Hydrochloric acid, besides being a digestive fluid in the stomach, as Prof Starling and Bayliss taught us reacting on the mucous membrane of the duodenum makes a secretion which causes the external secretion of the pancreas Owing to the experiments of Dr Nishina and Dr Yukawa the sulphate ion serves as an accelerator for the movement of the stomach and the intestines and, as a result, it serves to stir up their contents Also one can insist with the support of facts, that the hydrochloric acid makes a chemical component, and the sulphuric acid makes a mechanical component of the digestive process, and thus they complete the digestion

In preparing a laxative in treatment of kakke (beri beri) in Japan sulphate of magnesia with hydrochloric acid has been used for some time following the recommendation of Dr Ise, who found this useful combination as a result of his clinical observations of many years This combined employment of sulphate of magnesia and hydrochloric acid should not, therefore, be abandoned without due consideration

As regards the action of the phosphate ion on the duodenal wall, a few words here may not be out of place It reduces the amount of blood sugar, and causes the glycosuria to vanish This action is feasible in the case of a normal healthy person, but it is marked in the case of diabetes In other words, the phosphate

ion, acting on the duodenal wall, will stimulate the internal secretion of the pathologically weakened pancreas.

Particulars of this phenomenon have been supplied by the study of Dr Yoshiki of our laboratory

I would here show how the idea came to us, which guided us in the undertaking of these experiments concerning the anti-diabetic action of the phosphate ion, and a possible relation between the duodenal wall and the sugar metabolism.

We have at first taken into consideration what Prof. Pflüger contended namely, that damage to the nerve plexus of the duodenal wall of the frog, caused glycosuria, and again we have made observations on patients with parasites (strongyloides, found in southern parts of Kyushu which inhabit the duodenal wall) suffering from glycosuria, and moreover Dr Kanegae of our laboratory inserting a rubber balloon into the duodenum of the human being and causing it to expand for an hour to observe the duodenal movement, noticed the appearance of glycosuria by mere accident, and now again we observed that, when the stomach and the intestinal wall were massaged, the level of blood sugar was raised the highest when the duodenal wall was touched

From the view point of these phenomena we have assumed the existence of a close relation between the duodenal wall and the appearance of glycosuria, and, in this belief we repeated the experiments

It was always found that by injecting various extracts especially from the duodenal wall into the vein, the blood sugar was raised

If, again, acids or salts of several kinds were injected subcutaneously or intravenously, the ascent of the blood sugar could only be noticed

Next, when any of the mentioned substances were imported directly into the duodenal lumen, also the blood sugar was observed to rise higher, when, however, phosphate of soda was imported, the blood sugar was reduced In particular, a mixture of acid phosphate of soda (NaH_2PO_4) and phosphate of soda solution (Na_2HPO_4), when giving the reaction of pH 7 3–7 1 was found most effective Next we dissolved 10 grms of the phosphate of soda in 120 c cs of water, by taking this solution internally or by sending it directly into the duodenum of the human being by means of a tube the decline of the blood sugar was proved This was so marked in the case of patients suffering from diabetes, that even the disappearance of glycosuria was observed

While there is no doubt as to this being *the joint action of the phosphate ion and the duodenal wall on the sugar metabolism*, how and where the substance so acting has been formed is now under investigation

The report of Dr Elias' school that the injection of phosphate of soda into the vein of patients suffering from diabetes resulted in the disappearance of glycosuria, while the taking of the same medicament internally might cause a

contrary effect because plenty of phosphate of soda given internally may produce a diarrhœa, conflicts with the results of our experiments, it is possible that their *conditions of observation might have been different from ours*

Next, according to the results obtained by Dr Nishio of our laboratory, *during the observation of the absorption of quinine from the intestinal lumen, salts of cholic acid protect the intestinal wall, so as to lubricate its absorbing process* He also observed that if chininum hydrochloricum be put in the small intestine of the dog, it not only interferes with the absorption of the quinine that has been put in for the second time, but will also markedly hinder the intestinal wall from absorbing glucose, salt and water

When however, bile is added, this hindrance is neutralized

For the small intestine of a dog, a dog's bile is the most efficacious, taurocholate of soda being almost as potent as dog's bile, while glycocholate of soda is found to be feebly potent If a colloid fluid be applied instead of bile, still a more or less protective action is noticeable, the neutralizing action of bile against the inhibiting process of quinine may be due to its content of salts of cholic acids on one side and to its being a colloid solution, perhaps, on the other side

Moreover concluding with regard to the experiments in the test tube we believe the hardly soluble quinine base forms an easily soluble compound with salts of cholic acids If the clear solution of chininum hydrochloricum is made alkaline with any alkali the quinine base is precipitated, while the solution becomes turbid When a small quantity of bile is added to this turbid solution, the solution again becomes transparent, the precipitate being dissolved

Summary

On the basis of our own experiments, we have come to believe that the *sulphuric acid ion coming into contact with the mucous membrane of the stomach,* forms a substance resembling peristaltic hormone and makes the mechanical component of digestion, while the hydrochloric acid and pepsin make the chemical components, salts of cholic acids protect the intestinal wall, completing the absorbing function, phosphate of soda acting on the duodenal wall manifests its action by stimulating the internal secretion of the pancreas

In addition, these anions might possess many other physiological and patho*logical actions, but here we are confining ourselves to those which we have* been able to demonstrate

DISCUSSION

Lieut -Col R N Chopra, I M S (Bengal) Said he had listened with great interest to Dr Onodera's paper The work was on new lines which have not received great *attention in the past It was known that* intravenous injections of $MgSO_4$ produced diuresis and diarrhœa but the mechanism had not been properly studied It was very interesting to hear of the sugar reducing property of the phosphate ion and the action of massage on the duodenal wall.

Dr S L Sarkar (Bengal) Wanted to know the nature of the pharmacological action, whether it was based on the hormonic theory as well as the theory of blood sugar formation

Dr N Onodera (Japan) replied We do not know how the phosphate ion acts It has been noticed that if the drug is given by the mouth no action is induced It should be placed just on the first part of the duodenum in order to produce its effect on the blood sugar

SECTION VI.

VETERINARY.

ANIMAL INFECTIOUS DISEASES AND THEIR CONTROL IN JAPAN.

BY

NORICHIKA NAKAMURA, D.A.
Veterinary Laboratory, Nishizahara, Tokio

THOUGH there are several kinds of animal infectious diseases in Japan, I will in my present paper deal only with the control of some of them which are prevailing at present and have been studied in the past few years

In the first place, I may make a few remarks on *infectious pleuro pneumonia of cattle*

Although our country had been quite free from this disease till the second quarter of 1924, in September the first three cases of the disease were discovered among Mongolian cattle imported from Dairen, China, at the Yokohama Animal Quarantine Station The disease was fortunately stamped out there, the infection not being introduced into the interior of the country In the latter part of May, next year, sudden appearances of this disease were observed in a dairy farm in the city of Osaka The disease spread not only to the vicinity of the farm but over five neighbouring prefectures In the same year, some other cases of the disease were discovered among the imported cattle from Chosen where the occurrence of this disease has been stationary since 1922 In both times, all the animals exposed to infection as well as the affected have been slaughtered

According to the descriptions concerning the Animal Diseases Act, the complete eradication of the disease can only be obtained by the killing of all animals which are supposed to harbour the virus in their bodies But, as you know, it is hardly possible to kill all animals which are supposed to be affected without causing a great loss to the stock industry Moreover, the isolation or quarantine of the suspected animals for a long period has often met with great difficulty in practice For this reason I and one of my colleagues, Futamura, have endeavoured to study several methods of serological diagnosis that is the agglutination, precipitation and complement fixation tests, for the early detection of this disease in living

animals and finally we have found that the complement fixation test is most reliable for this purpose if specific and potent antigen be used

Our antigen was made as follows —A ten days' old culture on three per cent horse serum agar (three per cent) was emulsified in 3 c cs of salt solution after removal of the condensation water and the emulsion was heated at a temperature of 60° (Celcius) for three hours and mixed with 0 5 per cent solution of carbolic acid If 0 2 c c of the preparation does not exceed one half to one fourth the anti complementary dose determined on testing the antigen is suitable for use

The presence of precipitinogen in the sera of naturally infected animals seems to be closely related to the presence of virus in the circulating blood Moreover, the presence of precipitinogen in the liquefied necrotic foci and that of the Ziegler s ' perivasculare organisationsherd ' in the interstitial tissue as well seem to be very important for the diagnosis of this disease especially in the face of other ambiguous materials for instance, tuberculosis From the results of a considerable amount of work on the complement fixation test in which our antigen has been employed we came to the conclusion that this test can be carried out in practice at the animal quarantine station for the detection of affected animals among imported cattle from certain localities where this disease is spread

This test would also be applicable I think for the herds in localities where the occurrence of this disease is observed

In the next place, I should like to make a few remarks on *infectious diseases of hogs*

The prevalence of infectious diseases of swine has caused a great deal of loss in the pig raising districts of our country It has been long believed that the occurrence of several diseases of swine namely swine cholera swine erysipelas and swine plague could be demonstrable as a single disease without any mixed infection among them

Swine plague is very widely spread at present causing great losses among sucklings In some localities swine erysipelas is prevailing with varied severity in a limited enzootic extension and not infrequently epizootically Swine cholera invades often into such infected herds with varying intensity causing several cases of mixed form So it is obvious that the preventive measures must be based on exact ætiological observations otherwise any favourable results can not be expected at all

According to my long observation natural infection of pigs from swine plague, which occurs sooner or later after weaning may probably be due to the organism existing in the soil this being favoured by factors lowering the natural resistance of the animals I therefore attempted to confer immunity on animals, and supposed that vaccination might be more effective in sucklings than in adults, if properly carried out The practical application of this measure as I expected, reduced very much the outbreaks of this disease Therefore, I may say vaccination of sucklings can be recommended as an effective method of eradicating the disease

The vaccine used for this purpose is prepared by almost the same method as described by Wassermann and Citron using a number of strains of *B suisepticus* which have been isolated from lesions From my experiences it may be said that the vaccination should be carried out three times by the time of weaning in doses of 0 2 0 3 and 0 5 c cs respectively I have applied this vaccinotherapy to the disease and obtained in a number of cases favourable results This treatment will therefore be worthy of application for curative purposes to noble breeds affected with this disease

For practical measures against *swine erysipelas* both the simultaneous method with serum and culture and serum alone have been employed for many years But it has been noticed that the vaccination itself can probably be responsible for the spreading of this disease notwithstanding that the effectiveness of Lolenz s protective inoculation has been positively established

An attempt has been made by Fujimura a member of our laboratory to prepare a safe and effective vaccine by treating the organism of swine erysipela with iodine and very favourable results were obtained Since 1924 this vaccine as well as immune serum has been used practically in certain districts where this disease was prevalent The duration of the immunity established by this vaccination may be expected for about half a year and such an iodized vaccine when properly kept retains its potency for about one month

Inasmuch as the outbreaks of *swine cholera* may be checked and the spreading of this disease to surrounding herds may be prevented by serum inoculation the protective measures against swine cholera are aided effectively by the vaccination The vaccine used is an emulsion of the spleen and liver from an affected hog being mixed with carbolized glycerinated water in the proportion of one to one to two

This preparation was made by H Futamura in our laboratory and has been used in our country since 1920 the protective results having been practically proved to be satisfactory The immunity produced by this treatment lasts at least for six months and the vaccine retains its potency for about two or three months when kept in the ice chest at 5° (Celcius)

Finally I may read some remarks with respect to *infectious diseases of poultry*

With the increase of poultry keeping there has also been an increase in the occurrence of poultry infectious diseases which have not been demonstrated up to this time in our country Now the number of cases of fowl cholera fowl typhoid fowl pox and diphtheria bacillary white diarrhoea coccidiosis tuberculosis and fowl pest is increasing and these are regarded as the important infectious diseases in poultry

Fowl cholera is a widespread disease for many years in our country, causing very heavy losses Great care is being exercised by poultrymen for the prevention of this disease The application of the serum is in our country indicated only to flocks already infected or to those which are threatened by infection With a view to carrying out effective control of the disease attempts have been made

in our laboratory to prepare suitable vaccines from cultures Some reliable results have hitherto been obtained by the use of polyvalent nucleo proteids which produce a solid immunity in animals From the point of view of the veterinary police it is as you know very important to distinguish fowl typhoid from chronic fowl cholera

I expect however that the prevention against fowl typhoid by other means than immunization for which the inoculation of serum alone or killed culture is essentially concerned will probably prove unsuccessful

Fowl pox and diphtheria including so called roup are widely spread among our poultry farms the latter especially used to cause very severe losses in our poultry industry Both troubles have been considered by some investigators to be caused by the same agent I have noted however in several experiments that fowl pox and fowl diphtheria are two different diseases to be thoroughly distinguished from each other My attention was drawn to the possibility that the causal agent of fowl diphtheria might not be any filterable virus From my further examination it was found that the cocco bacillus isolated from the local lesions in the earlier stage of fowl diphtheria played some important role as a causal agent of this disease In association with this finding I should like to emphasize that the results of the treatment with the immunized serum against this organism proved to be uniformly favourable in the prevention of the disease The serum thus prepared is therefore applicable for the practical control of this disease Recently my attempt to protect chickens against infection for a long time (about a few months) has proved to be favourable by the inoculation of the sero vaccine which has been prepared by mixing the immune serum with culture in a proportion of one to five

Although *coccidiosis* is one of the widespread diseases in our country we have at present no idea regarding the control of the disease It was about five years ago that the existence of *bacillary white diarrhœa* caused by *B pullorum* was demonstrated in our country According to extensive investigations over a couple of years it was shown that this disease was already widespread over the land and inflicted severe losses upon the poultry keepers As regards the method of eradicating this disease the germ carriers should be distinguished by employing the agglutination test From our experiments it was found that for a positive diagnosis one must keep to above one to fifty in the complete reaction But it must be pointed out that the application of this measure is commonly limited to the infection carrying adult hen From our examinations we can say that all the cocks giving positive results in the blood tests should be removed from a breeding flock In one case I and Y Kawamura one of my colleagues have proved the presence of the germ in the testicles This fact leads us to suppose the possibility of occurrence of infection in eggs laid from a non reacting hen

Finally the occurrence of *fowl pest* must not be neglected Since the first case of this disease was found by Dr Migita chief inspector of Osaka Quarantine

Station, in 1925, attempts have been made to inquire into its propagation in our country and several cases have been demonstrated in certain localities I am, therefore afraid that even at the present time cases of this disease may have been treated as an unknown disease There has been a number of descriptions concerning the susceptibility of pigeons to the virus of fowl pest, but in our present knowledge it appears to be still doubtful whether this is true or not From our experiments with respect to this question, it has been confirmed that the susceptibility of the pigeon differs according to the age of the individual, very young and very old pigeons are less susceptible, while those weighing about 300 grms are most susceptible Moreover, it was found that successive passage of the virus through pigeons is quite possible the death of the animal occurring constantly five to six days after the appearance of the paralytic symptoms Having examined the blood of the affected pigeons, it was found that, within three to four days before the appearance of the symptoms, a remarkable difference in percentages of various leucocytes took place, the number of lymphocytes decreased, no such changes having ever been found in the blood of fowl cholera Recently, trials in treatment of this disease with vaccine originating from various organs of affected pigeons or chicks, have been made Though no definite conclusions can yet be drawn from our work vaccination seems to be promising I think, it is very probable that the successful control of this disease will be dependent upon the production of an effective immunization

In conclusion, I may express my best thanks for your friendship in giving me an opportunity to stand here before you to read this paper

DISCUSSION

Mr J T Edwards (United Provinces) There are several points in Dr Nakamura's comprehensive paper which strike the worker familiar with the livestock diseases of India One of these is the marked difference in incidence of the great epizootics For example the essayist has not mentioned bovine hæmorrhagic septicæmia, the second most important infectious disease of cattle in India Again, he has paid much attention to contagious bovine pleuro pneumonia, a disease which, from the information available, does not exist in India I have often wondered what is the significance of this fact one knows very well that pleuro pneumonia is a very serious and widespread affection of cattle over a large part of the African continent and also occurs in the Far East, while India in her intervening position for some reason has escaped infection *Is there* any risk of the disease becoming established in the country through the importation of affected cattle from the countries where it exists ?

To some extent the circumstance that Japan is a temperate country over a large part of its surface may account for the difference in the kind and incidence of prevalent diseases However, this cannot be the reason for the difference in incidence of contagious bovine pleuro pneumonia, for the disease occurs with marked severity, as has been said in the warm countries of Africa There also occur very commonly in India outbreaks of pleuro pneumonia among sheep and goats, the causal nature of which has not yet been accurately determined

At Muktesar, we have made endeavours to repeat accurately the work of certain Japanese authors, as recorded recently in the *Japanese Journal of Veterinary Science*, upon the preparation of a vaccine for preventive inoculation against rinderpest, but we have not been able to demonstrate any protective action in the material when it has been used upon our highly susceptible hill cattle

It is of interest to note the attention paid to fowl diseases in Japan Recently, outbreaks of a disease which bears some resemblance to fowl pest have caused severe ravages in northern India

BOVINE TUBERCULOSIS IN INDIA

BY

J T EDWARDS,

Imperial Institute of Veterinary Research, Muktesar

THE general impression has been that tuberculosis is so rare among cattle in India that it hardly merits attention In Western countries it is one of the most besetting problems of the livestock breeder and the veterinary scientist About 30 per cent of the cattle are infected and the degree of clinical morbidity among the infected animals is often very severe Added to this there is the public health aspect of the problem a very large proportion of certain forms of human tuberculosis notably the abdominal tuberculosis of children bone and joint tuberculosis cervical gland tuberculosis is caused by tubercle bacilli of the bovine type, taken into the body mainly by the ingestion of milk from infected animals

If the assumption is correct therefore that bovine tuberculosis is actually rare in its incidence in India at the present time and in addition, circumstances exist in the country for its dissemination in the manner of Western countries the most drastic measures ought to be taken by State authorities to prevent risk of introduction of the disease with imported infected cattle

The number of careful examinations upon cattle for the detection of tuberculosis that have been actually conducted in this country have not been many but so far as they have gone they would seem to corroborate the general impression that tuberculosis is rare A few years ago examinations of carcases of slaughtered cattle at the Ferozepore (Taylor) and Cawnpore (Oliver) slaughter houses revealed macroscopic lesions of tuberculosis in approximately three per cent of the animals the lesions were invariably however, minute in extent localized to the tissues in which they are most commonly found and retrogressive in their appearance Tuberculosis had also been detected in Madras among conservancy bullocks (Krishnamurthi) Bombay Presidency (Sowerby a somewhat severe case) and in the Calcutta Jail from the results of tuberculin tests apparently (Kerr) The Imperial Dairy Expert Mr W Smith in 1923 however, declared that from an experience extending over many years among military dairy cattle all over India he was unacquainted with the disease Curiously in the cold weather of 1923–24 a deputation of Indian students sent by me under one of my officers to ascertain the incidence of the disease at the Lahore and Ferozepore slaughter houses, failed

to discover lesions in carcases subjected to an ordinary examination such as is commonly performed in meat inspection

Such information as was, therefore, available indicated that the disease was rare, at any rate so rare that it would hardly seem to merit attention from any actual losses caused by it among livestock in India, but nevertheless of importance, in that its introduction among the livestock population might lead eventually to the appearance of a degree of almost uncontrollable morbidity simulating that of the West, if timely steps were not taken to avert this trend of development It has been said on authority (McFadyean) that State measures could not be faced in Europe for economic reasons against the disease until the natural incidence fell through some other agency below about 10 per cent In India we would seem to be, from the information available well below this threshold of effective action, and it would be incumbent upon veterinary authorities therefore to recommend drastic action by extermination of infected animals in the face of all diagnosed infection in India, and further to suggest quarantine measures for the exclusion of infected cattle at the ports of landing

Laboratory examinations to determine the so called types of tubercle bacilli that are responsible for the various kinds of lesions in human tuberculosis in India have not yet been conducted upon a scale to warrant from them even speculation as to the common origin of infection Work of this kind has now been accomplished in Europe to such an extent that one has fairly accurate knowledge as to the types responsible for the various classes of lesions The most common forms especially of adults, with pulmonary lesions are almost invariably caused by the human type of bacillus whereas forms that are abdominal in origin especially the common abdominal form of children and the so called surgical forms of tuberculosis are very frequently caused by bacilli of the bovine type At the Second Conference of Veterinary Officers in India in 1923 Hutchinson made the important announcement that the incidence of the forms a high proportion of which is known in Europe to be caused by bacilli of the bovine type bears in India almost exactly the same numerical relationship to the other forms as has been calculated to exist in Europe *A priori* this statement would seem to point in the direction of infection of human beings to a very grave degree with tubercle bacilli of bovine origin In any event, both from the livestock and public health standpoints investigations at the laboratory into the incidence and nature of infection among cattle in India would appear to be strongly indicated In 1923 therefore, the Indian Research Fund Association was approached to furnish me with trained collaboration at Muktesar to elucidate systematically the various aspects of the problem, and I was fortunate in securing the services of Dr M B Soparkar for the work Some of the experiments already completed have been published under his name, while much of the work remains as yet unrecorded

Two questions of fundamental importance had to be resolved at the commencement of the enquiry —(1) Are Indian cattle more resistant to tuberculosis than are cattle of European origin ? (2) Are the tubercle bacilli that give rise to the

relatively mild tuberculosis occasionally detected among cattle in India of lower virulence than the bacilli isolated from the grave lesions of cattle in Europe?

Some work carried out previously by Glen Liston and Soparkar in Bombay seemed to indicate that indigenous calves showed sometimes a much greater resistance to artificial infection than what is known to be possessed by European calves inoculated in the same manner Sheather, however, at Muktesar concluded that cultures *obtained from the lesions of Indian cattle* were *decidedly* less virulent for both small laboratory animals and cattle than strains of European origin as Soparkar has pointed out, there were some defects in the technique of the experiments conducted by Sheather

Calves from the most important breeds of cattle from all over India, calves containing a considerable admixture of European blood (as partial controls) and buffalo calves of various breeds were tested at Muktesar by subcutaneous inoculation (of 50 milligrams) of a *young culture of a strain kindly forwarded by* Dr Stanley Griffith from England for the purpose and declared by him to be fully virulent The results of this large test were to some extent inconclusive there were no pure bred European calves, of the requisite age used concurrently in the test However a large proportion of the calves succumbed to acute generalized *infection* within *three months* in the same manner as calves in Europe are known to succumb after this kind of test (Royal Commission on Tuberculosis), the buffalo calves proved as susceptible as the other calves A considerable number of the calves nevertheless displayed a high degree of resistance and recovered from the test, among these calves was also a fair number of the calves with the admixture of European blood

It might be concluded from the results of such an experiment that a noteworthy *proportion* of Indian cattle possess a *hereditary resistance against* tuberculosis which distinctly surpasses that observed in European cattle As will be mentioned later, we have now some reason to believe that the mechanism of resistance among such animals can be explained on other than hereditary grounds

Strains of tubercle bacilli isolated by Dr Soparkar from the restricted lesions *observed in Indian cattle* proved, *after appropriate animal inoculation to be fully* as virulent as control strains of highly virulent bovine bacilli obtained from Europe The results are communicated by Dr Soparkar in another paper to this Meeting

It would therefore, seem that a good reason to explain the low natural incidence of bovine tuberculosis in India is to be found in the common mode of life of cattle in the country *One knows that the rarity of tuberculosis among sheep and goats* in Europe is not attributable to any naturally greater resistance of these animals as compared with cattle but to their common method of maintenance—upon open pastures throughout their lives—in circumstances where massive propagation of infection from animal to animal, as occurs frequently among housed cattle has little chance to occur Indeed we have now *further experience to illustrate that* when cattle are maintained in India in conditions of domestication simulating those of the West a high incidence of clinical tuberculosis may develop in a herd

after introduction of infection (Quetta military dairy) The prevailing infection among indigenous cattle as disclosed by the laboratory tests would appear to be no less virulent than that of Europe its dissemination among animals would thus take place after voidance from carriers in circumstances which would render infection operative only through the medium of minimal doses probably frequently repeated so that the existence of a preceding infection within the tissues would set up a vaccinating effect against each succeeding dose of virulent material

We have now further information which lends considerable support to this hypothesis and moreover makes it necessary to recast some of our former impressions concerning the relatively low incidence of natural bovine tuberculosis in India and the mechanism of resistance among some cattle in the country towards artificial infection

In the course of very searching examinations carried out by Dr Soparkar last cold weather at the Lahore and Ferozepore slaughter houses, he found macroscopic evidence of tuberculosis in over 15 per cent of the carcases of cattle I will be glad if he will supply any more exact figures he can recall in connection with this investigation in the course of the discussion in nearly all these cases the tuberculous nature of the lesions could be confirmed by detection of the bacilli or histological examination

Further it has been known to us for some time that after the application of the tuberculin test to what had been taken to be healthy cattle in the course of the work, there has sometimes appeared a disconcertingly high proportion of apparent reactors In such animals usually no lesions are found on slaughter Dr Soparkar has been able to isolate fully virulent tubercle bacilli from the tissue material of some animals of this kind

I understand that in the United States of America likewise the problem of what is known as no lesion tuberculosis has become very prominent in the course of the extensive work now undertaken there to clear herds of the disease upon the so called 'accredited herd system It is not unlikely that further work will prove that the high resistance possessed by some cattle in India towards artificial infection arises from the circumstance that infection of this kind is relatively widespread, for a latent infection is well known to set up a vaccinating effect among the animals that harbour the infection

Incidentally, the information that has become available concerning the high proportion of animals in apparent good health that there may be affected with tuberculosis of the obscure lesion' or probably of the no lesion' kind and in the carcases of which a diagnosis of tuberculosis would be most likely missed entirely by a man who was not highly expert at the work of detection makes it necessary for us to recast our views also somewhat upon what was believed to be the relative unreliability of the ordinary subcutaneous tuberculin test as applied to Indian cattle The disturbingly high proportion of reactors among tested animals was attributed to some defect in the test itself notably the liability of Indian cattle to considerable diurnal fluctuations in temperature It is not unlikely that the

reactions in these cases were not fallacious but indicative of obscure or latent infection of the kind now described To circumvent the difficulty which we believed to attach to the subcutaneous test, we have carried out a considerable amount of work on the applicability of certain local tests—the intra palpebral test, and the double intradermal test (Medical Research Council) The Muktesar Institute has already circularized veterinary workers in India upon the last named test the methods of employing it upon cattle, the kind of tuberculin for use, and *its reliability for current diagnosis in* expert hands

We have not yet encountered any cases of macroscopic affection of the udder with tuberculosis It has been proved by certain European workers that virulent bacilli may be excreted by an infected cow with the milk even when the udder shows no obvious disease and that they are also commonly excreted with the dung

Having regard to the great public health and agricultural significance of the problem concerning which some facts have been lightly touched upon in the course of this short paper it seems to me to be of the utmost importance that researches should be pressed forward upon it by both medical and veterinary authorities working in close collaboration The problem of experimental tuberculosis research in man and animals is decidedly a single one Among other work accomplished in the course of this enquiry already is the examination of tuberculosis found in other species of animals in India—horses, swine, camels elephants We have reason to *believe that horses are affected with both bovine and avian types of bacilli here* Swine have been found by Dr Soparkar to be affected in the few cases he has examined from Bombay with the human type pre eminently, and also with the bovine type He has found an example of mixed bovine and avian infection in cattle and of a cervical tuberculosis caused by the avian type of bacillus in man

VIRULENCE OF TUBERCLE BACILLI ISOLATED FROM CATTLE IN INDIA

BY

M B SOPARKAR, M D,

In Charge, Bovine Tuberculosis Inquiry, Indian Research Fund Association

From the Imperial Institute of Veterinary Research Muktesar

TUBERCULOSIS has been generally held to be a rare disease among cattle in India and the rarity of the disease has been attributed by Sheather (1921) to a relatively low degree of virulence in the causal organism

The results of his experiments at Muktesar led him to conclude that 'they appear to indicate beyond all possibility of doubt that the strains of tubercle bacilli infecting cattle in India possess a distinctly lower degree of virulence than tubercle bacilli isolated from cattle in Europe'

These conclusions were based mainly upon the results of inoculation of rabbits and Indian calves with culture of a strain of tubercle bacilli isolated from a cow from the abattoir at Ferozepur

Experiments upon rabbits had shown that when these animals were inoculated intravenously with 0 1 or with 0 01 milligram of this strain they lived longer than is usually the case when strains of European origin are employed comparison being made with the results obtained by the Royal Commission on Tuberculosis (1907)

Indian calves consisting of ten buffaloes and twelve bull calves were inoculated subcutaneously half the number with 50 mg and the other half with 10 mg of culture of the Indian strain and none of the animals died of the disease as a result of the infection On the other hand in the experiments previously carried out by Liston and Soparkar (1917) upon Indian calves with a strain of tubercle bacillus of European origin in the same doses a proportion of calves had died of acute generalized tuberculosis and the remaining ones had survived the observation period and had shown comparatively limited and non progressive lesions

On looking into the details of the methods of investigation employed, it appeared that the results obtained by Sheather were due to an old culture employed by him for the tests It is stated in the report (page 40) that the inoculum was obtained

from a number of broth flasks that had been in the incubator for six months and in which a small island of growth, an inch or so in diameter, had appeared *A portion of the growth was removed and allowed to drain on the side of* a test tube for a day before it was used for purposes of inoculation It is probable that many of the bacilli in the culture after such a prolonged incubation and exposure for draining might have been dead or so devitalized as to render *the effective dose of living vigorous bacilli much smaller in the* apparent amount actually employed for inoculation That prolonged incubation leads to the gradual death of an increasing number of tubercle bacilli in culture is supported by long laboratory experience of various workers Calmette (1923) observes *that old cultures of six or more months, reproduce themselves only exceptionally* However they can still infect susceptible animals although the development of lesions may be very slow In general after eight to ten months almost all the bacterial elements are either dead or so devitalized that they can no longer be revived

The results recorded by Sheather therefore might probably have been due in reality to a comparatively small dose of living and vigorous bacilli than to any marked attenuation in the virulence of the strain employed For even in the more susceptible European cattle although large doses of 50 mg of virulent bovine tubercle bacilli derived from a young culture produce an almost invariably fatal acute generalized tuberculosis and medium doses of 10 mg produce variable results small doses are often known to produce only a slight disease Cobbet (1917) summarizing the results of numerous experiments carried out for the Royal Commission on Tuberculosis observes 'Small doses of bovine tubercle bacilli whose virulence was proved by control experiments produced when injected into calves only localized or limited lesions which soon became fibrous or calcareous and thus assumed a retrogressive type, while the animals themselves after a transient disturbance of health remained in excellent condition up to the time when they came to be slaughtered and examined'

The results of the experiments in which six months' old broth cultures were used cannot therefore be strictly compared with those recorded by the Royal Commission on tuberculosis who always used young vigorously growing serum cultures for test inoculations upon animals nor with those obtained by Liston and Soparkar (1917) who also used young 30 days' growth on solid media for inoculating their animals

On *a priori* grounds, therefore the conclusions arrived at by Sheather do not appear to be based on sufficiently controlled experiments As the subject, however was of considerable importance and the conclusions drawn connoted a fundamental *biological difference* in the character of the bacilli infecting animals in the two countries and also because tubercle bacilli of low virulence have been found infecting animals of some species experiments were undertaken to ascertain the correctness of this view

A strain was obtained from Dr A S Griffith through the Lister Institute of Preventive Medicine and it was declared by him as highly virulent Previous experiments with this strain employed as a young three weeks' growth for infection of rabbits had shown that a dose of 0 1 mg injected intravenously produces generalized tuberculosis with death within 18 to 37 days and a dose of 01 mg in 23 to 45 days (Table I)

TABLE I

Result of inoculation of rabbits with a virulent bovine tubercle culture of European origin (G 14) (Intravenous)

Number of Rabbits	Dose in Milligrams	Duration of Life in Days		
		Maximum	Minimum	Average
11	0 1	37	18	22
12	0 01	45	23	30

The same strain was then planted on broth and the flasks kept in the incubator for six months The culture was then prepared for inoculation of animals by the same method as that adopted by Sheather for his experiments with the Indian strain

Four rabbits were inoculated intravenously with 0 01 mg and four others with 0 1 mg of the culture thus prepared The details are shown in Table II It will be seen that one of the rabbits inoculated with 0 01 mg died in four days of intercurrent disease and another in 36 days of internal hæmorrhage The other two rabbits died of uncomplicated tuberculosis one in 45 days and the other in 89 days after inoculation *The four rabbits inoculated with 0 1 mg died of generalized tuberculosis* in 25, 31 34 and 42 days respectively In Sheather's experiments the two rabbits inoculated with 0 1 mg died of generalized tuberculosis, one in 36 and the other in 43 days Of the two other rabbits inoculated with 0 01 mg, one died of generalized tuberculosis in 50 days and the other in 70 days after inoculation

TABLE II

Intravenous inoculation of rabbits with a six months' old broth culture of highly virulent bovine tubercle bacilli (European strain G 14)

Number of Rabbits	Dose in Milligrams	Weight of Rabbit in Grammes		Duration of Life in Days	Post mortem Result *	
		Initial	Final		Naked eye Appearance A	Microscopic Examination B
586	0 01	1,100	890	D 4	Died of intercurrent disease	
600 A	0 01	900	770	D 45	Lungs ++++ Liver +++ Spleen +++ Kidneys + Lymph glands ++	+ + – + +
606	0 01	950	1 030	D 36	Lungs ++ Liver – Spleen – Kidneys + Lymph glands –	+ – Died of internal – hæmorrhage. + –
514 D	0 01	1 750	1 100	D 89	Lungs ++++ Liver +++ Spleen – Kidneys ++ Lymph glands ++	+ – –
515 D	0 1	1 500	1 200	D 25	Lungs +++ Liver + Spleen ++ Kidneys + Lymph glands +	+++ + + + +
516 D	0 1	2 250	1 900	D 34	Lungs +++± Liver ++ Spleen ++ Kidneys ++ Lymph glands ++	+ – + Died of + internal hæmorrhage

TABLE II—*concld*

Number of Rabbits	Dose in M lb grams	Weight of Rabbit in Grammes		Duration of Life in Days	Post mortem Result *	
		Initial	Final		Naked-eye Appearance A	Microscopic Examination B
517 D	0 1	1 500	1 000	D 42	Lungs ++++ Liver +++ Spleen ++ Kidneys ++ Lymph glands +++	+++ + + + +
518 D	0·1	1 800	1 110	D 31	Lungs +++ Liver − Spleen − Kidneys ++ Lymph glands ++	 + ++

* *Note* A —

− No macroscopic tuberculous lesions
+ A few do do do
++ A fair number of do do
+++ Numerous or extensive tuberculous lesions
++++ Very numerous or very extensive tuberculous lesions

D — Died

Note B —

− No tubercle bacilli detected
+ A few tubercle bacilli detected
+± Some do do do
++ Many do do do
+++ Numerous do do do
++++ Very numerous do do do

The close similarity between the two series of results recorded above suggests that the Indian strain employed by Sheather was in all probability not of very low virulence

Further experiments were made upon rabbits with the same virulent strain employing a culture grown upon serum agar but incubated for six months The details are shown in Table III One of the two rabbits inoculated with 1 10 mg died of intercurrent disease and the other died of generalized tuberculosis after 98 days The length of life of the others inoculated with 1 100 mg was 89 and 121 days respectively In another experiment the same culture after incubation for six months was removed from the media and kept in a covered sterile watch glass on the laboratory bench protected from the direct rays of the sun, but exposed to diffused daylight for a day before being used for inoculation

TABLE III

Experiments on rabbits with a six months' old culture of a virulent bovine tubercle strain of European origin (G 14) (Intravenous)

Description of culture inoculated	Number of Rabbit	Dose in Milli grams	Weight of Rabbit in Grammes.		Duration of Life in Days	Post mortem Result *	
			Initial	Final		Naked eye Appearance	Microscopic Examination
The culture was incubated for six months and inoculated soon after removal from the media	6[illegible] B	0 1	1 580	1 6[illegible]0	D 11	Died of intercurrent disease	
	29[illegible] B	0 1	1 810	1 260	D 98	Lungs +++ Liver + Spleen + Kidneys ++ Lymph glands ++ Intestine ++	+++ +++ +++ +++ − +++
Do	[illegible]91 C	0·01	1 0[illegible]0	8[illegible]0	D 89	Lungs ++++ Liver + Spleen + Kidneys ++ Lymph glands ++ Heart ++	++ +++ +++ +++ +++
Do	578 B	0 01	1 400	1 000	D 1[illegible]1	Lungs ++++ Liver − Spleen Kidneys+ Lymph glands −	++ − − +
The same culture incubated as above but exposed for one day to diffused daylight after removal from the medium	154 B	0 01	1 1[illegible]0	800	D 59	No evidence of tuberculosis	
Do	[illegible]92 A	0 01	1 [illegible]00	1 500	D [illegible]49	No evidence of tuberculosis	
Do	581 B	0 1	1 [illegible]0	1 390	D 119	Lungs contained a few scattered caseous nodules No other evidence of tuberculosis	
Do		0 1	1 1		D 54	No evidence of tuberculosis	

* See note II

Died.

t

Two rabbits were inoculated with 1 10 and two with 1 100 mg of this culture *intravenously* Of the former one died in 51 days but showed no evidence of tuberculosis the other died after 119 days with only a few scattered caseous nodules in the lungs and no visible lesions elsewhere Of the latter one died in 59 and the other after 249 days and no evidence of tuberculosis was detected in the animals

The results indicated that a large majority of the bacilli in the culture were probably dead The deleterious effect upon tubercle bacilli of exposure to light has been demonstrated by several workers and also by the author (Soparkar 1917) and results of this experiment afford an additional evidence

As to the virulence of the Indian strain for Indian calves Sheather found as has been stated previously that whereas in the experiments carried out by Glen Liston and Soparkar on Indian calves with a bovine strain of European origin some of the calves inoculated with 50 mg of culture died of acute generalized tuberculosis none of his animals inoculated with the same dose of the Indian strain died or developed a progressive tuberculosis An experiment was therefore carried out also upon Indian calves Six calves three of hill breed and three obtained from Bareilly were inoculated subcutaneously with 50 mg of the same strain of highly virulent bovine tubercle bacilli obtained from Europe but grown upon broth incubated for six months and prepared in the manner adopted by Sheather for his experiments The culture was the same as that used for experiments upon rabbits mentioned previously which in fact were inoculated simultaneously along with the calves The details of the experiment are given in Table IV One of these animals died 25 days after inoculation Examination showed some minute tubercles in the lungs and the thoracic glands but the affection did not appear to the naked eye of sufficient severity to account for the death The rest of the animals lived for a long time remained in good condition and gained considerably in weight After more than a year these animals were used for other experiments and when examined on death they showed only a small lesion at the seat of original inoculation whilst in two cases slight tuberculosis of the nearest lymph glands alone was noticed In two cases no trace of even the local lesion could be detected These results are in marked contrast to those of experiments previously conducted by the author (Soparkar 1926) in which when care was taken to use young cultures containing living and vigorous bacilli the same strain had produced different results (*See* Table V) In these experiments out of 12 calves of hill breed inoculated eight died of acute generalized tuberculosis and four survived and showed only limited lesions while out of nine calves of Rohilkhand breed obtained from Bareilly five died of acute generalized tuberculosis and four survived and had developed only slight tuberculosis

The experiments so far conducted however afforded proof of only an indirect nature In order to obtain direct evidence as regards virulence of bovine tubercle bacilli of Indian origin attempts were made to study the characters of tubercle bacilli obtained from Indian cattle

TABLE IV.

Experiments on calves with a six months' old broth culture of a virulent bovine tubercle strain of European origin (G 14) (Subcutaneous) Dose 50 mg

Number of Animal	Age in Months	Weight in Pounds	Duration of Life in Days	Gain (+) or Loss (—) in Weight	RESULT
255	17	138	465		* Slight tuberculosis
256	22	137	771		* Slight tuberculosis
257	18	137	461		* No evidence of tuberculosis
226	13	119	610		* Do Do
227	8	119	D 25	—20	Generalized tuberculosis slight
229	4	147	603		* Localized tuberculosis

* The animals after having survived for a year were used for other experiments examination after death from these experiments revealed the lesions noted against each.

D — Died

Two strains were isolated from cattle slaughtered at the Cawnpore abattoir One was derived from a small pea sized lesion in the pharyngeal gland of a bullock and the other from a similar localized lesion in the bronchial gland of another bullock There was no other detectable lesion of tuberculosis in the bodies of these animals Very few tubercle bacilli were detected microscopically in one case and none in the other

The strains were isolated from guinea pigs inoculated with an emulsion of the caseous material from the glands These were designated Indian B I and Indian B II A number of guinea pigs were inoculated with these strains but it is not intended to draw any inferences from the results in these animals since most of them were injected with tissue emulsions of lesions in which the bacterial content was variable

TABLE V.

Experiments on calves with a virulent bovine tubercle strain of European origin (G 14) (Subcutaneous) Dose 50 mg

Number of Calves	Age in Months	Weight in Pounds	Breed	Age of Culture in Days	Duration of Life in Days	Gain (+) or Loss (−) in Weight	RESULT
109	5	105	Rohilkhand	23	D 101	−20	Generalized tuberculosis, moderate
110	5	122	Do	23	K 129	+28	Localized tuberculosis
111	6	110	Do	21	D 52	+ 1	Died of causes other than tuberculosis
112	6	141	Do	16 to 22	K 93	+19	Slight tuberculosis
113	6	120	Do	21	D 36	−20	Generalized tuberculosis, moderate
114	6	122	Do	21	D 26	−18	Generalized tuberculosis, moderate
115			Do				Died before inoculation
116	7	120	Do	21	D 34	+ 8	Generalized tuberculosis slight
117	5	60	Do	21	K 104	+38	Slight tuberculosis
118	8	100	Do	21	D 38	+ 6	Generalized tuberculosis, slight

D = Died K = Killed

TABLE V—*concld*

Number *of* Calves	Age in Months	Weight in Pounds	Breed	Age of Culture in Days	Duration of Life in Days	Gain (+) or Loss (—) in Weight	RESULT
219	5	197	Rohilkhand	21	K 95	—77	Slight tuberculosis
222	8	92	Do	21	D 25	+ 6	Died of causes other than tuberculosis
145			Hill	23	D 28		Generalized tuberculosis severe
146	5		Do	23	D 40		Generalized tuberculosis moderate
147	6	90	Do	21	D 50	—10	Generalized tuberculosis slight
148	6	101	Do	16 to 22	D 43	—26	Generalized tuberculosis slight
149	5	80	Do	21	K 103	+82	Slight tuberculosis
150	5	68	Do	21	K 103	—54	Slight tuberculosis
151	7	120	Do	21	D 60	—10	Generalized tuberculosis moderate
152	7	107	Do	21	D 19	+ 8	Generalized tuberculosis slight
153	7	138	Do	21	K 96	+12	Slight tuberculosis
154	7	82	Do	21	D 26	—7	Generalized tuberculosis slight
155	7	158	Do	21	K 96	+ 2	Slight tuberculosis
156	8	119	Do	21	D 21	—18	Generalized tuberculosis slight

D = Died K = Killed

A study of the cultural characters of these strains showed that both strains were typically dysgonic They produced, when planted upon plain serum agar a thin grey dryish looking film without wrinkling or any thick nodules When transplanted upon media containing glycerine (glycerine serum agar and glycerine egg) growth appeared to be very sparse and in many cases no growth developed on [illegible]

of incubation Virulence of these strains was tested upon rabbits The cultures were grown upon a solid medium (serum agar) and care was taken to use a young culture for the tests

The workers for the Royal Commission on Tuberculosis in their experiments to determine the virulence of strains used cultures after about three weeks of incubation In all experiments described here when different strains were examined in test doses for comparative virulence the cultures were incubated for a definite period of three weeks The growth was gently removed from the surface with a platinum loop and weighed in a sterile tared watch glass The growth was then thoroughly rubbed up in a fresh state and without undue exposure in a mortar at first with a drop of sterile bile which helped to break up the clumps and subsequently with physiological salt solution to make a homogeneous suspension which was further suitably diluted so that one c cm of it represented the required dose *The details of the tests upon rabbits are shown in Table VI*

It will be noted that the duration of life of one rabbit inoculated with 1 100 mg was much longer than that of the other This may be ascribed to a difference in the susceptibility of individual animals Cobbet (1917) discussing the results of inoculation of rabbits with strains of human origin observes 'The difference in the severity of the results could not be attributed to difference in the virulence of the viruses but so far as they could not be accounted for by differences of dose and mode of inoculation they could only be explained on the assumption that the individual rabbits differed much in susceptibility to tuberculous infection Griffith also found that individual susceptibility of rabbits exercises an important influence on the duration of life In the experiments under discussion the other animal inoculated with 1 100 mg of Indian B I died in 28 days and the other two animals inoculated with 1 10 mg died in 15 and 27 days respectively Of the animals inoculated with the other strain Indian B II those inoculated with 1 100 mg died in 21 to 27 days and those receiving 1 10 mg in 22 to 23 days of acute tuberculosis The results indicated that the strains were not of markedly low virulence

In order to obtain further data for comparison with results obtained by Sheather from experiments on cattle the strains were simultaneously tested on calves

Six calves of hill breed and six of Rohilkhand breed obtained from Bareilly and one calf of Hallikar breed from Southern India were used for inoculation with the strain Indian B II Similar batches of calves consisting of six hill cattle four

TABLE VI

Intravenous inoculation of rabbits with Indian strains of tubercle bacilli isolated from cattle

Designation of Strain.	Number of Rabbit	Dose in Milli grams	Weight of Rabbit in Grammes		Duration of Life in Days	Post mortem Result *	
			Initial	Final		Naked eye Appearance A	Microscopic Examination B
Indian B I	112 A	0 01	1,600	1,300	D 28	Lungs ++++ Liver ++ Spleen +++ Kidneys + Lymph glands ++ Heart ++	+++ + ++ + + ++
Do	343 A	0 01	1,650	750	D 92	Lungs +++ Spleen + Kidneys +	+++ + +++
Do	342 A	0 1	1,650	1,070	D 27	Lungs ++++ Spleen —	++++ ++
Do	349 A	0 1	1,550	1,160	D 15	Lungs ++++ Liver — Spleen —	· · ·
Indian B II	21 A	0 01	1,200	690	D 24	Lungs ++++ Liver — Spleen — Lymph glands +	++++ + + +
Do	22 A	0 01	950	650	D 27	Lungs ++++ Liver +++ Spleen ++ Kidneys + Lymph glands ++	·· · ·· ·
Do	247 A	0 1	1,100	800	D. 22	Lungs ++++ Liver — Spleen — Kidneys —	·· +++ ++
Do	583 A	0 1	1,430	890	D 23	Lungs ++++ Liver ++ Spleen — Kidneys — Lymph glands — Bone marrow	++++ + +++ + ++ ++

* *See* note under Table II. D = Died

Bareilly calves and one of Hissar breed were inoculated in a like manner with the strain Indian B I Each calf was inoculated subcutaneously on the left side of the neck with 50 mg of a three weeks' old culture of the Indian strain Out of four calves of Rohilkhand breed inoculated with Indian B I one died of complication in 19 days and another of progressive tuberculosis after 19 weeks and two survived Out of six hill calves two died of acute generalized tuberculosis, one in 29 and another in 31 days respectively and four survived Out of six Rohilkhand calves inoculated with the other strain Indian B II one died of debility within a few days after inoculation and the rest died of acute generalized tuberculosis in 36 to 64 days Of the other six calves of hill breed, four died of acute generalized tuberculosis in 18 to 236 days and two survived One calf of Hallikar breed also died of acute generalized tuberculosis in 45 days Comparison of these results with those obtained after inoculation of Indian cattle with a known virulent bovine strain of European origin can be made from data shown in Tables V and VII

TABLE VII

Experiments on calves with Indian strain of tubercle bacilli isolated from cattle (Subcutaneous) Dose 50 mg

Designation of Strain	Number of Animal	Age in Months	Weight in Pounds	Breed	Age of Culture in Days	Duration of Life in Days	Gain (+) or Loss (−) in Weight	RESULT
Indian B I	232	9	170	Hill	21	596		* Slight tuberculosis
Do	233	10		Do	21			*Surviving*
Do	235	12	100	Do	21	477		* Slight tuberculosis
Do	239	5	90	Do	21	D 31	−4	Generalized tuberculosis, slight
Do	240	18	116	Do	21	594		* Generalized tuberculosis moderate

* The animal having survived for 14 months was used for other experiments and the result mentioned was noted after death from these experiments and revealed the lesions noted against each.

D = Died K D = Killed dying

TABLE VII—*contd*

Designation of Strain	Number of Animal	Age in Months	Weight in Pounds	Breed	Age of Culture in Days	Duration of Life in Days	Gain (+) or Loss (—) in Weight	Result
Indian B I	241	5	76	Hill	21	D 29	—8	Generalized tuberculosis slight
Do	34	18	236	Hissar	21	K D 214	+44	* Localized tuberculosis
Do	223	12	98	Rohilkhand	21	D 149	—11	Generalized tuberculosis slight
Do	246	8	92	Do	21	588		* *Slight tuberculosis*
Do	251	7	140	Do	21	460		* Localized tuberculosis
Do	252	7	70	Do	21	D 19	—7	Slight tuberculosis
Indian B II	231	7	112	Hill	21	D 55	—22	Generalized tuberculosis moderate
Do	234	9	164	Do	21	D 18	—22	Generalized tuberculosis slight
Do	237	11	114	Do	21	D 236	+96	Generalized tuberculosis slight
Do	238	12	96	Do	21	592		* Localized tuberculosis.

* The animal having survived for 14 months was used for other experiments and the result mentioned was noted after death from these experiments and revealed the lesions noted against each

D = Died K D = Killed dying

TABLE VII—*concld*

[illegible]ignation Strain	Number fo Animal	Age in Months	Weight in Pounds	Breed	Age of Culture in Days	Duration of Life in Days	Gain (+) or Loss (—) in Weight	RESULT
[illegible]an B II	24[illegible]	10	109	Hill	21	D 45	—10	Generalized tuberculosis, slight
Do	243	6	66	Do	21	592		* Slight tuberculosis
Do	176	11	127	Do	21	D 45	—2	Generalized tuberculosis, slight
Do	2[illegible]8	8	115	Rohilkhand	21	D 64	—13	Generalized tuberculosis, slight
Do	230	8	95	Do	21	D 4	—5	Died of debility
Do	248	7	88	Do	21	D 36	—6	Generalized tuberculosis, moderate
Do	244	7	78	Do	21	D 57	—18	Generalized tuberculosis, slight.
Do	[illegible]45	9	89	Do	21	D 30	—6	Generalized tuberculosis, moderate
Do	2[illegible]3	5	53	Do	91	D 37	—4	Generalized tuberculosis, slight

* The animal having survived for 14 months was used for other experiments and the result mentioned was noted after death from these experiments and revealed the lesions noted against each

D = Died

Whilst the very variable susceptibility exhibited by Indian cattle to tuberculosis makes it difficult to institute a strict comparison between the degrees of virulence displayed by the two strains yet the fact that a number of animals inoculated with Indian strains died of acute generalized tuberculosis in the same way as those inoculated with virulent bovine virus of European origin clearly indicates that the Indian strains were not of markedly low virulence, although some individual difference was noted between the two Indian strains in their effects when inoculated into calves

A further proof of the high virulence of the Indian strain was obtained when one out of three calves inoculated with even a small dose of 5 mg of culture of Indian B II died of acute generalized tuberculosis in 47 days after inoculation (Table VIII)

TABLE VIII

Subcutaneous inoculation of calves (Rohilkhand breed) with an Indian strain of bovine tubercle bacilli (Indian B II) Dose 5 mg

Number of Animal	Age in Months	Weight in Pounds	Age of Culture in Days	Duration of Life in Days	Gain (+) or Loss (—) in Weight	RESULT
310	4	105	21 days	D 41	—25	Generalized tuberculosis slight
307	9	105	Do	481		* Localized tuberculosis
309	6	87	Do	511		* No evidence of tuberculosis

D = Died * *See* note under Table VII

Subsequently strains of tubercle bacilli isolated from cattle in other provinces have been investigated with a view to ascertaining if differences exist in the virulence of tubercle bacilli affecting cattle in different parts of India

Two strains were isolated from cattle at Muktesar One of these was from an apparently healthy buffalo which had given a marked reaction to double intradermal tuberculin test and on examination after death had shown tuberculosis of the thoracic glands and some affection of the lungs The strain was isolated

through a guinea pig and was found to be markedly dysgonic in cultural characters It was tested on rabbits and the results are noted in Table IX The other was from a bull which was presumably healthy but had given a positive reaction to the subcutaneous (thermal) tuberculin test Post mortem examination did not reveal any naked eye lesions of tuberculosis in the body, but when a congested lymph node which appeared otherwise normal was excised and its emulsion inoculated into animals they developed tuberculosis in about four weeks A strain was isolated from one of these animals This was also dysgonic in character and was *tested on rabbits The details are shown in Table X*

TABLE IX

Intravenous inoculation of rabbits with a strain of tubercle bacilli isolated from a buffalo (Muktesar B II.)

Number of Rabbits	Age and Generation of Culture	Dose in Milligrams	Weight of Rabbit in Grammes		Duration of Life in Days	Post mortem Result *	
			Initial	Final		Naked eye Appearance A	Microscopic Examination B
133	21 days old 2nd generation	0 01	2 460	1 700	D 36	Lungs ++++ Liver ++ Spleen +++ Kidneys ++ Lymph glands +	
134	Do	0 01	1 8?0	1 500	D 24	Lungs +++ Liver Spleen — Kidneys — Lymph glands — Bone marrow —	+++ ++ ++ + ++ ++
135	Do	0 1	1 660	1 400	D 25	Lungs ++++ Liver — Spleen + Kidneys — Lymph glands + Bone-marrow	++++ +++ +++ + +++ ++
136	Do	0 1	1 760	1 120	D 21	Lungs ++++ Liver — Spleen + Kidneys — Lymph glands + Bone-marrow	+++ + + + ++ ++

D = Died * See note under Table II

TABLE X

Intravenous inoculation of rabbits with Indian strain of tubercle bacilli isolated from a bull which showed no naked eye lesion of tuberculosis (Muktesar B I)

Number of Rabbits	Age and Generation of Culture	Dose in Milligrams	Weight of Rabbit in Grammes		Duration of Life in Days	Post mortem Result *	
			Initial	Final		Naked eye Appearance A	Microscopic Examination B
129	24 days old 1st generation	0 01	1 670	1,250	D 30	Lungs ++++ Liver + Spleen +++ Kidneys ++ Lymph glands + Bone marrow –	+++ ++ ++ + ++ ++
130	Do	0 01	1,810	1,450	D 36	Lungs ++++ Liver ++ Spleen +++ Kidneys – Lymph glands +++	 · ·
131	Do	0 1	1 590	1 400	D 22	Lungs ++ Liver + Spleen ++ Kidneys – Lymph glands – Bone marrow –	++++ ++ ++ + ++ +++
132	Do	0 1	1,420	1 250	D 23	Lungs ++++ Liver – Spleen – Kidneys – Lymph glands – Bone marrow	++++ +++ +++ + +++ ++

D – Died * See note under Table II

The result of tests of these two strains showed that all the animals died within three to five weeks of acute general tuberculosis, those receiving 1 10 mg within 21 to 25 days and those inoculated with 1 100 mg in 24 to 36 days

No material difference was noted in the result when compared with that obtained with a European virus It is worthy of note that the strain recovered from one of these animals which did not even show any naked eye lesion of tuberculosis was fully virulent indicating that the restricted nature of the lesions (in this particular instance there was hardly any lesion at all) was not due to low virulence of the infecting organism as has been suggested by Sheather He states, 'A point which would appear to support the view that the lower virulence of the organism is the more important factor is that in practically every instance the natural

lesions which have come under observation have been restricted to a few glands and have been to a very large extent calcified '

Further observation on a number of strains from cattle from different provinces are in progress but a summary of the results of examination of some of the strains isolated from animals that were found to be suffering from generalized tuberculosis may be recorded here Five strains were isolated from conservancy bullocks belonging to the Madras Corporation The material was obtained through the courtesy of the Principal, Madras Veterinary College, and Mr V Krishnamurti Ayyar, Professor of Pathology and Bacteriology at the college Two of these strains, both from the same animals, but derived from different tissues were found to be mixtures of avian and bovine bacilli The three remaining strains were found to be of the bovine type The virulence of these was tested on rabbits Four animals were inoculated with each of these strains by the intravenous route two with 1 10 mg and two with 1 100 mg The results are shown in Table XI In all cases the rabbits (except one that died two days after inoculation of intercurrent disease) died of acute generalized tuberculosis, those inoculated with 1 10 mg in 19 to 21 days and those receiving 1 100 mg in 25 to 35 days , the results closely resembling those obtained from inoculation of a highly virulent bovine virus of European origin

TABLE XI

Experiments of rabbits with Indian strains of tubercle bacilli isolated from cattle showing generalized tuberculosis (Intravenous)

Designation of Strain	Number of Rabbit	Age and Generation of Culture	Dose in Milligrams	Weight of Rabbit in Grammes		Duration of Life in Days	Post mortem Result *	
				Initial	Final		Naked eye Appearance A	Microscopic Examination B
Madras B I	29	21 days old, 2nd generation	0 01	2,220	1,700	D 35	Lungs ++++ Liver − Spleen − Kidneys ++ Lymph glands −	++++ − − + +
Do	30	Do.	0 01	1,600	1,250	D 33	Lungs ++++ Liver ++ Spleen ++ Kidneys + Lymph glands −	++ + − −
Do	31	Do	0·1	1 600	1,550	D 19	Lungs +++ Liver + Spleen +++ Kidneys − Lymph glands	+ + +

D = Died * See note under Table II

TABLE XI—*concld*

Designation of Strain	Number of Rabbit	Age and Generation of Culture	Dose in Milligrams	Weight of Rabbit in Grammes		Duration of Life in Days	Post mortem Result *	
				Initial	Final		Naked eye Appearance A	Microscopic Examination B
Madras B I	32	21 days old, 2nd generation	0 1	1,900	1,400	D 20	Lungs ++++ Liver ++ Spleen ++ Kidneys − Lymph glands −	++ + + − −
Madras B II	33	Do	0 01	1,800	1,500	D 35	Lungs ++++ Liver − Spleen − Kidneys + Lymph glands +	++ − − + +
Do	34	Do	0 01	1,750	1,570	D 2	Died of intercurrent disease	
Do	35	Do	0 1	1,450	1,300	D 20	Lungs ++++ Liver ++ Spleen + Kidneys − Lymph glands −	++ + +
Do	36	Do	0 1	1,600	1,160	D 24	Lungs ++++ Liver + Spleen + Kidneys ++ Lymph glands ++	++++ ++ +++ ++ −
Madras B IV	37	Do	0 01	1,350	1,220	D 25	Lungs ++++ Liver +++ Spleen ++ Kidneys + Lymph glands −	++ ++ ++ ++ ++
Do	38	Do	0 01	1,870	1 470	D 35	Lungs ++++ Liver − Spleen +++ Kidneys +++ Lymph glands	++++ ++ +++ +++
Do	39	Do	0 1	1,600	1 400	D 23	Lungs ++++ Liver + Spleen ++ Kidneys ++ Lymph glands ++	++ + ++ + +
Do	40	Do	0 1	1 640	1,400	D 20	Lungs +++ Liver − Spleen − Kidneys − Lymph glands −	++ − − − −

D = Died

* *See* note under Table II

Summary

Bovine tuberculosis is generally held to be a rare disease in India and this has been attributed to a lower virulence of the organism affecting cattle in India as compared to the virulence of tubercle bacilli isolated from cattle in Europe

The tendency of the lesions detected in Indian cattle to remain restricted to a few glands and their calcification have been adduced as evidence in support of this view

The experimental results on which this view is based were found to have been obtained by a method of investigation which is shown to yield similar results with even a highly virulent strain of bovine tubercle bacilli of European origin Indian strains of tubercle bacilli isolated from cattle when tested by methods in which certain fallacies were eliminated showed that they were fully as virulent as tubercle bacilli obtained from cattle in Europe

Tubercle bacilli isolated from a gland of a bull without any naked eye lesion were found to be as fully virulent as those obtained from cattle that were suffering from generalized tuberculosis

The rarity of gross tuberculous lesions hitherto observed among indigenous cattle and the usual tendency of the lesions to remain localized in affected animals cannot therefore be ascribed to any noteworthy difference in the virulence of the infecting organisms

I wish to express my deep gratitude to Mr J T Edwards Director Imperial Institute of Veterinary Research Muktesar for much valuable help and the facilities afforded for carrying out the work at the Institute Appreciation is also due to Sub Assistant Surgeon Jemadar Chanchal Singh Dhilon I M D, for his valuable assistance

References

Calmette A (1923) Tubercle Bacillus Infection and Tuberculosis in Man and Animals English translation by Soper and Smith p 53

Cobbet L (1917) The Causes of Tuberculosis Cambridge University Press pp 61 and 361

Griffith A S (1907) Roy Com Tuberculosis *2nd Int Report, App Vol I* p 439

Liston, W G and Soparkar, M B (1917) The Susceptibility of Indian Milch Cattle to Tuberculosis *Ind Jour Med Res* Vol V, p 19

Royal Commission on Tuberculosis (1907) 2nd Int Report Part II App Vol I p 437

Sheather, A L (1921) Virulence of Tubercle Bacilli isolated from Bovine Lesions in India *Memoirs of the Department of Agriculture in India* Veterinary Series Vol. III No ?

Soparkar M B (1917) The Vitality of the Tubercle Bacillus outside the Body *Ind Jour Med Res* Vol. IV, p 627

Idem (1926) The Relative Susceptibility of Indian Milch Cattle of Various Breeds to Tuberculosis *Ibid*, Vol XIII p 755

DISCUSSION

R S Dr Debakar Dey (Bengal) I endorse every word which fell from the lips of Mr Edwards and Dr Soparkar I would only refer to a paper headed 'Tuberculosis' which I had the honour to read on Sunday, the 7th December, 1924, in connection with the Bengal Health Association, held under the presidency of Rai Bahadur Dr Gopal Chandar Chatterjee I am inclined to believe that the bovine tuberculosis, human tuberculosis and avian tuberculosis are one and the same, only behaving differently in different environments I respectfully ask the President and the bacteriologists at Muktesar whether their recent work agrees with my findings or not

Mr V Krishnamurti Ayyar (Madras) I should like to say a few words in connection with this subject

In a paper contributed by Glen Liston and Soparkar in the *Indian Journal of Medical Research* Vol V, 1917-18, entitled 'The Susceptibility of Indian Milch Cattle to Tuberculosis' they have stated as follows 'In the opinion of the Superintendent, Civil Veterinary Department, and the Principal, Madras Veterinary College, Madras, tuberculosis is not known to exist among cattle in Southern India and both attribute this to the same cause namely, the open air life of the cattle' Glen Liston and Soparkar were, however, of a different opinion and thought that it was possible that the freedom of cattle from tuberculosis might be due to the natural immunity or a relatively greater resistance to the disease than European or American cattle and conducted some experiments the conclusions of which are stated as follows —

'The experiment confirms the general experience that Indian cattle are less commonly affected by tuberculosis than English cattle are and supports the view that the comparative infrequency of the disease among cattle in India is due to a natural resistance rather than to any method of housing or keeping cattle in India as compared with England

The next work in India was done by Sheather and the conclusion which he arrived at, as Soparkar has pointed out in his paper, was that the strains of tubercle bacilli infecting cattle in India possess a distinctly lower degree of virulence than tubercle bacilli isolated from cattle in Europe

The rarity of tuberculosis in India has been attributed therefore by one set of workers to the natural resistance possessed by Indian cattle and by the other to the low virulence of tubercle bacilli

In this connection, I would like to mention that in a disease like tuberculosis, individual susceptibility plays a great part whether in small laboratory animals or bigger experimental animals and I may mention that even the results of the few experiments which are now in progress at the Madras Veterinary College appear to confirm this In the case of small laboratory animals, the duration of life is cut short or prolonged, and, in bigger animals, either a progressive or retrogressive form of tuberculosis occurs, depending on their susceptibility Griffith found that the individual susceptibility of animals exercises an important influence on the duration of life and that on many occasions an animal inoculated with a smaller dose died before the one inoculated with a larger dose of the same culture

Hutyra and Marek state that 'the fact that tuberculosis occurs more frequently in some breeds than in others is in a great measure due to the different conditions under

which they may be kept or the uses to which they may be put Cattle from the Russian steppes for example become infected just as readily as the more domesticated when they are removed from pasture and fed in stables'

They also state that in experiments on artificial infection it has been noted that among animals of the same breed and age some resist infection with large masses of virulent bacilli It would therefore appear to be difficult to base any definite opinion on the results of experimental inoculations alone whether low incidence of bovine tuberculosis in India is due to the natural resistance of cattle or to the low virulence of the bacilli

In Madras the first case of tuberculosis was recorded by me in 1916 in one of the bullocks belonging to the Corporation and since then the incidence among them seems to have been on the increase In them the lesions present have invariably been wide spread involving the lungs, kidneys intestines mediastinal and mesenteric glands In a paper read by me at the Indian Science Congress held at Lahore this year which embodied the results of the post mortem examinations held on cattle of the Madras Corporation I stated that the rarity of tuberculosis among cattle in this country appears to be due more to the mode of life in which they are kept than to other reasons and that, when they are placed in the same conditions as the Corporation cattle of Madras the problem of tuberculosis infection would be as severe as that found in Western countries

In this connection I may also add that Hutyra and Marek state as follows —

'In the open air the danger of infection is in general very slight The bacilli which are expelled with the expired air of diseased animals are rapidly disseminated and are destroyed in a short time after reaching the ground by the action of sunlight Accordingly tuberculosis is almost unknown among animals that are continually on pasture'

Dr Norichika Nakamura (Japan) Are there any noticeable differences between Indian cattle and European cattle in the tuberculin test for the detection of the disease?

We have found many cases of swine tuberculosis among the slaughtered animals at Tokio and the bovine type of the tuberculosis bacillus has been chiefly demonstrated in the lesions The lesions were localized in the lymphatic glands at the neck (maxillary lymphatic glands)

Dr P V Gharpure (Bombay) If bovine tuberculosis is responsible for producing in man a bone and gland type of tuberculosis and if pathological evidence based on post mortem examination in man can be taken as proof of the type to tubercle present I feel inclined to think that the bovine form is extremely rare and bovine tuberculosis plays a very minor part in the discrimination of this infection in man The total frequency of tuberculosis as judged from over 6 000 post mortems on man works out at about 25 per cent and over 90 per cent of these belong to an acute ulcerating form due to one may say direct infection by inhalation.

Col A J Williams R A V C (B India) The cases demonstrated showed a marked difference in the extent and severity of the lesions in Indian calves inoculated with European bovine virus Can Dr Soparkar explain this?

Dr M B Soparkar (B India) replied With regard to the incidence of tuberculosis among indigenous cattle to which reference was made by Mr Edwards, I have

recently found that in certain localities it is prevalent to a greater extent than has hitherto been recorded Examination of carcasses at the slaughter houses at Ferozepur and Lahore has shown an incidence of over 16 per cent, and in 14 per cent of the animals examined (over 600) tubercle bacilli were demonstrated microscopically With regard to the instances cited by Dr Gharpure of the failure of the guinea pig to develop tuberculosis after inoculation with material in which tubercle bacilli were detected, such *instances have also been met with by several investigators* This may be due to the bacilli in the material being dead or in rare cases to the possibility of the bacilli belonging to some other type to which the animal may not be susceptible This point is discussed *in another paper before the Congress*

With regard to the question raised by Col Williams as to the cause of the high resistance shown by some of the calves we are not in a position, in the present state of our knowledge, to give any particular reason for the phenomenon It is not known for certain whether it is due to a natural immunity or to an acquired resistance Some recent experiments, as yet unpublished, have shown that when presumably healthy cattle were tested by the subcutaneous tuberculin test a large proportion of them (75 per cent in one series) showed a positive thermal reaction In view of the general opinion *held about the rarity of the disease among cattle, it was considered improbable that* such a large proportion of them could have been infected Post mortem examination on many of them failed to reveal any naked eye evidence of tuberculosis and the large number of apparently positive reactions was considered to be due to wide diurnal variations in the temperature of the animals However, in one instance, when an apparently non tubercular gland from one of these reacting animals was excised and animal inoculations were done from it, the animals developed tuberculosis showing that the reacting bull was infected with tuberculosis This indicates the possibility that the high resistance displayed by some of the animals may be due to previous slight infection conferring a certain degree of immunity As to the cross bred animals many of the calves with an admixture of European blood were found in the experiments on the susceptibility of Indian cattle to be no less resistant than the indigenous animals One batch of these animals was obtained from a place where Johne's disease was very prevalent Whether this circumstance had any connection with the very high resistance displayed by the animals it *is difficult to say.*

Mr J T Edwards (United Provinces) replied Mr Krishnamurti's views as quoted from the concluding portion of the paper read by him before the Indian Science Congress coincide with those expressed in my paper in so far as he now believes the very low [illegible] here loss in India is *attributable mainly to the usual mode of life* [illegible] rches the conclusions drawn in the earlier [illegible] and Soparkar, Sheather), referred to by him at some length, may now be largely disregarded He also stresses the part played by individual susceptibility in the genesis of morbid infection Differences in this respect among animals of the same species are well known to workers upon experimental tuberculosis, but it is doubtful whether this factor is of greater significance in tuberculosis than it is generally in infectious diseases

Dr. Nakumura asks whether I have any special remarks to make upon the tuberculin testing of Indian cattle I have dwelt upon this matter briefly in the course of my

paper Earlier experience tended to indicate that the ordinary or subcutaneous test was frequently not reliable on account of the large number of thermal reactions observed in what appeared to be healthy cattle More recent information would seem to make one hesitate to accept such cattle as entirely non infected, for we have had some evidence to show that infection with tuberculosis which gives rise to no visible lesions or to merely obscure lesions is not uncommon in India However, our work upon other forms of tuberculosis testing which depend upon the production of specific local swellings at the seat of injection of the tuberculosis shows that in experienced hands such tests can yield readily the desired diagnostic information in the usual conditions of maintenance of Indian cattle We have recently recommended the so-called double intradermal test (of the Medical Research Council) for this purpose There is not infrequently some difficulty in adjudging whether the local swelling is indicative of a tuberculosis reaction or not We have found—what has been stressed by the originators of the method—that the potency of the tuberculin plays an important part With a very strong tuberculin, one may find difficulty in interpreting the significance of swellings which occur in a large proportion of the animals although when a considerable number of animals are done in a herd, the degree of swelling in the actually infected animals can usually be distinguished from that which occurs in most of other animals, which are presumably healthy

There is no reason to believe as has been suggested by Col Williams, that animals which contain an admixture of European blood are more susceptible to tuberculosis than are indigenous cattle

AN IMPROVED VACCINE FOR IMMUNIZATION AGAINST RINDERPEST

BY

MAJOR R A KELSER, V C,
U S Army Medical Department Research Board, Manila, P I

WITH THE CO OPERATION OF
DR STANTON YOUNGBERG

AND

DR TEODULO TAPACIO,
Bureau of Agriculture, Manila, P I

RINDERPEST is an acute, febrile, contagious disease of cattle, carabaos (water buffaloes) and occasionally other ruminants It is caused by an ultra microscopic filterable virus

At the present time this disease is extensively prevalent in most of Asia It also occurs, to a lesser extent, in various parts of Africa and, in Europe, in the southern portion of the Balkan peninsula

It is believed that rinderpest was first introduced into the Philippine Islands in 1886 or 1887, presumably from Indo China It has since spread to various of the islands of the archipelago and to date has taken a toll in animal lives amounting to hundreds of thousands This malady is by far the most serious of all diseases of livestock prevalent in the Philippines As the late Governor General Wood stated, in certain sections of the islands it destroys so many animals as to interfere seriously with the economic development of the people

In countries where rinderpest has been a problem, a vast amount of work has been done with a view to finding a cure for the disease or a satisfactory means of immunizing susceptible animals against it

Following reports that the Boers had, for a long time, obtained good results with bile in immunizing animals in the Transvaal against rinderpest, Koch(1) in 1897, investigated their claims He found that when bile from cattle dead of rinderpest was administered in rather large amounts to susceptible animals, it afforded them considerable protection against subsequent infection Koch's observations were soon confirmed by Kohlstock(2) Kolle(3), Theiler(4), Turner(5) and a number of others

Edington(6) recommended the addition of one part of glycerin to two parts of bile when using the latter for immunization purposes Then, in order to produce a

more substantial immunity, Kohlstock(7) recommended giving animals which had received the bile treatment 0 2 cubic centimeter of virulent blood about two weeks subsequent to the injection of bile

The bile, and bile and virulent blood methods of immunization against rinder pest have been used on a rather large scale and up to the time better methods were developed they served a useful purpose

Back in 1893 Semmer(8) reported that the blood of animals which had recovered from rinderpest possessed considerable merit as an immunizing agent This finding was not taken advantage of on any appreciable scale until some years afterwards However the observation was later confirmed by a number of investigators Further it was demonstrated that a more potent serum could be produced by administering increasing amounts of virulent blood to immune animals used for serum production Thus, the use of anti rinderpest serum became an important factor in the treatment and prevention of the disease

In the treatment of rinderpest it was soon found that in order to obtain favourable results with serum, it must be administered very early in the disease and be given in relatively large amounts

As an immunizing agent it was found that as with most serums, the immunity conferred is of but short duration This led Kolle and Turner(9) and several others to introduce the simultaneous method of immunization In this method susceptible animals are given an injection of anti rinderpest serum and such inoculation immediately followed by an injection of 1 or 2 cubic centimeters of virulent blood

The simultaneous method of immunization against rinderpest has, without question, been of great value in controlling rinderpest It has, however several distinct disadvantages In the first place a certain percentage of animals receiving this treatment develop rather severe reactions and some losses occur as a direct result of vaccination Then reacting animals are readily capable of spreading rinderpest to susceptible animals if contact is afforded, hence this feature must always be given due consideration when the simultaneous method of immunization is contemplated

In 1916 17 Boynton(10) working with rinderpest in the Philippines carried out a series of experiments to determine the infectiousness of various tissues from animals dead of rinderpest Among the experimental animals used in connection with this work, he had several head of cattle which had been injected with tissue preparations in which the virus had either died out or had become reduced in virulence to the point where it would not produce disease On subsequently inoculating these animals with virulent blood, Boynton found that they resisted the infection with little or no reaction This finding immediately suggested the possibility of a vaccine, prepared from the various tissues, as a satisfactory means of immunizing cattle and carabaos against rinderpest

For some years following this observation Boynton devoted his time to the development of such an immunizing agent and finally succeeded in producing a

vaccine of considerable merit In 1920 the Philippine Government put t
vaccine into use on a large scale It has proved of great value in rinderp
control work in the islands

As prepared by the Insular Bureau of Agriculture, Boynton's vacc
consists of a heated, glycerinized and phenolized mixture of blood and finely grou
tissues (spleen, liver kidneys lymph glands, heart and testicles) taken from anim
destroyed in the acute stages of rinderpest The blood content makes up from o
third to one fourth of the bulk and the proportion of phenolized glycerin addec
equal to one third the weight of the blood and tissue The reaction of the glyce
used should be about pH 7 8 The mixture is heated for three hours in a wat
bath maintained at 42° C For use this concentrated vaccine is diluted wit
fluid consisting of 33½ per cent glycerin in physiological saline solution

Kakizaki(11) and his associates Nakanishi, Oizumi and Nakamura of
Institute for Veterinary Research of Chosen Japan have done a large amount
work with rinderpest vaccine and report excellent results in the control of the dise
with vaccines prepared from various tissues of animals affected with rinderpest

In his early work Kakizaki employed an emulsion of spleen tissue in wh
the virus had been killed by prolonged contact with glycerin The handicap w
this vaccine was the long period of time required for its preparation Later t
investigator and his associates used heat and such chemicals as phenol tolu
alcohol ether, iodine and eucalyptol in their vaccine experiments Of these age
eucalyptol and toluol gave encouraging results

In the last report received Kakizaki and his co workers are destroying t
rinderpest virus in their vaccine through the combined action of heat, glycerin a
toluol Two to three parts of glycerin and 8 to 10 per cent toluol are added to t
tissue mixture which is then incubated at 37 5° C for seven to ten days

While toluol is undoubtedly of value in preparing rinderpest vaccine, we ha
found that its use results in a very viscid preparation which offers considera
difficulty in administration Further, absorption of toluolized vaccine is very slo

In the Philippines the vaccine prepared according to Boynton's method h
without question been of very definite value However there are several facto
in connection with its production which have proved to be quite serious In t
first place after preparation the vaccine must be allowed to age at refrigerat
temperature for a period varying from one to as much as five or six months before
can be used without danger of producing rinderpest Then after it has reached t
point where it can be safely injected it must be used within a relatively short peri
of time as it loses its potency as an immunizing agent rather rapidly The seriou
ness of these shortcomings is obvious First no appreciable surplus of vaccin
ready for use can be maintained on hand because of its poor keeping qualitie
Then, in the face of a serious outbreak of rinderpest it is impossible to quick
increase the vaccine output because of the long period of time required to proper
age the product Thus it is obvious that before the effects of an increase
production can be realized an outbreak of the disease can have spent itself

In February 1926 the United States Army Medical Department Research Board, at the request of the late Governor General Wood, undertook a study of the rinderpest vaccine prepared by the Bureau of Agriculture with a view to improving it. As a result of this investigation, the Board, working in co-operation with the Insular Bureau of Agriculture, has been able to produce a potent rinderpest vaccine which can be safely used within several hours after preparation. Immunization experiments carried out with a relatively large number of cattle and carabaos have fully established the value of this vaccine as an immunizing agent. While the tests to determine the duration of potency are not all complete, indications to date are that its keeping qualities are excellent.

Vaccine Prepared by the Medical Department Research Board

After working with various chemical agents we found that when chloroform was added, in proper proportion, to a mixture of blood and finely ground tissues from an animal killed in the acute stages of rinderpest, the virus was promptly destroyed. It was further ascertained that such mixture could then be safely used as an immunizing agent with excellent results.

Our next step was to determine whether or not all of the tissue constituents entering into the preparation of the vaccine were of value. Work along this line definitely demonstrated that the blood constituent was of no value as an immunizing agent. It was thus obvious that vaccines containing blood were being diluted with an inert agent which, because of its nature, detracted very materially from the keeping qualities of the vaccine. In so far as the various tissues are concerned, it was found that vaccines prepared from the lymph glands, spleen, liver, and from a mixture of kidney and testicular tissues, respectively, were all effective as immunizing agents.

As a result of these findings the blood was omitted in the preparation of our vaccine. Further, of the various lymph glands entering into the vaccine we found that the mesenteric glands frequently contain pathogenic, spore bearing organisms (*C œdematis-maligni*, *C tetani*, etc.) In order to eliminate possible trouble from such source, the mesenteric glands were omitted in the preparation of our vaccine.

After discarding the blood as a constituent of the vaccine, its consistency was changed to such an extent as to offer difficulty when it came to injection. This condition was overcome by adding sterile physiological saline solution to the ground tissues.

Our method of preparing the vaccine is as follows —

Susceptible cattle are inoculated subcutaneously with 2 to 10 cubic centimeters of fresh, citrated blood from an animal in the acute stages of rinderpest. The infected animals usually develop temperature evidence of rinderpest on the third day following inoculation. A day or two later the characteristic diarrhœa sets in together with other manifestations of acute rinderpest. With the onset of diarrhœa the temperature starts to drop. At this point the animals are bled to

death and with aseptic precautions the spleen, liver, kidneys, testicles, and various glands (sub maxillary, pre scapular, inguinals, etc) are removed and placed in sterile covered containers The mesenteric glands are, for reasons previously stated, omitted

The collected tissues are then conveyed to the laboratory and as much of the fat and fasciæ as possible carefully trimmed from them The organs are next placed in a 5 per cent solution of phenol for 15 minutes and then carefully rinsed in two changes of sterile water The tissues are then cut in pieces of convenient size to put through a large, sterilized food chopper, the ground material being collected in a sterile container as it comes through the machine This ground tissue is then carefully covered to prevent contamination and placed in the ice box at a low temperature (2° C) and allowed to remain until the following day After thus standing the tissue grinds better

The next step is to put the ground material through a specially constructed grinder which will grind the tissue so fine that most of the mass can be readily worked through a forty mesh sieve In using this grinding machine it is sterilized and the tissues put through it five or six times, grinding a little finer each time With the aid of a sterile pestle this finely divided tissue is immediately worked through a sterile, forty mesh sieve into a sterile, tared container The weight of the tissue is ascertained and for every gram of it 1 cubic centimeter of sterile physiological saline solution is added and a thorough mixture made This is then placed in sterile stock bottles and 0 75 per cent chloroform added and the bottles tightly stoppered and well shaken The bottles are then dipped in a 10 per cent solution of liquor cresolis compositus and stored in the refrigerator The chloroform destroys the rinderpest virus within several hours and is not detrimental to the immunizing constituent of the vaccine In most of our experiments the vaccine has been used within 48 hours after preparation

The grinding of the tissue is an important part of the process First the tissue must be finely divided to permit the chloroform to quickly destroy the rinderpest virus Then a finely divided state is essential in order that the finished product may be readily injected without clogging the syringe needle

It has also been our practice to shake the bottles of vaccine several times during the period they are in the refrigerator, for the purpose of distributing the chloroform, which settles to the bottom, through the tissue mass

We are also careful not to contaminate the necks of our stock bottles when filling same with the virulent tissue mixture Then as an added precaution, after adding the chloroform and just before inserting the stopper we swab the inside of the necks of the bottles with chloroform Obviously, the purpose of this is to eliminate the possibility of trouble from virulent material which might get between the neck of the bottle and the stopper and escape the action of the chloroform

While we have fully demonstrated that this vaccine can be safely injected within two or three hours following its preparation we have observed that it is better to allow it to stand 48 hours or more before use If injected within two or

three hours after preparation, a slight irritating effect results from the chloroform. While this is in no way serious it can be avoided by allowing the vaccine to stand a day or two

Tests of Vaccine Prepared by the Medical Department Research Board

During the first five or six months of our investigations various attempts were made to develop a means by which we could promptly destroy the rinderpest virus in tissue and blood mixture without injuring the material as an immunizing agent. This work involved the use of varying degrees of heat, and the use of such chemical as glycerin, toluol, phenol, and finally chloroform In our early endeavours, in addition to work with straight mixtures of tissue and blood, we tried to develop filtrates of tissue extracts and also precipitates which could be used as immunizing agents The results of such efforts were all more or less unsatisfactory until we developed our chloroform treated vaccine

The first test of our chloroform treated vaccine was carried out with four head of cattle In this and in all of our subsequent work with cattle, the animals used were imported direct from the Island of Fuga by the Bureau of Agriculture Rinderpest does not occur on the Island of Fuga so consequently cattle from there are all susceptible to the disease

In this test three of the four animals were each given three subcutaneous injections of 10, 15 and 15 cubic centimeters, respectively, of vaccine, an interval of one week elapsing between the injections The fourth animal served as a control and thus received no vaccine Ten days subsequent to the administration of the last dose of vaccine, all four animals were given 2 cubic centimeters of virulent rinderpest blood subcutaneously The following Chart indicates the results —

CHART 1.

Three of the four head of cattle included in this chart each received three injections of vaccine prepared from a mixture of tissue and blood The doses were 10, 15 and 15 c cs respectively, administered at weekly intervals One animal control

Animal No	Date vaccination completed	Date infected	Amount of virus	RESULTS
6214	Aug 28, 1926	Sept 7, 1926	2 c cs	Slight reaction Survived.
6215	"	"	"	No reaction Survived
6222	"	"	"	No reaction Survived
6219	CONTROL	"	"	Developed severe case acute rinderpest. Would have died but killed to conserve tissues.

Thus in this test all three of the vaccinated animals survived the infection, two with no reaction whatever the third with a slight temperature reaction The control promptly developed an acute case of rinderpest and would have died from same but in order to utilize the tissues for the preparation of more vaccine, it was destroyed when in the proper stage of the disease for vaccine production

Test to determine the Relative Value of Blood and other Tissues for the Production of Vaccine

The first step towards determining the value of the various ingredients entering into the preparation of this vaccine was to make three separate lots of vaccine from given group of animals infected for the purpose One lot was prepared from blood alone a second lot from a mixture of the various organs while the third lot was made in the usual way and represented a combination of the blood and organs

Twenty head of cattle were used to test these three lots of vaccine They were divided into two groups of ten each In one group four animals were each given three doses of the vaccine prepared from the blood alone, four were treated in a similar manner with vaccine prepared from the combination of various organs and the remaining two served as controls In the other group eight of the ten head of cattle were each given three doses of the vaccine prepared from the mixture of blood and organs, the remaining two animals serving as controls for this group

The dates of vaccination were the same for both groups of animals However the group which received the vaccine prepared from the mixture of blood and organs was infected two days after the other group As it was our intention to utilize all animals which developed rinderpest for vaccine production, we infected the two groups on different dates in order that we would not have more animals than we could handle in a given time should a large percentage of them develop the disease

Chart 2 depicts the results obtained with the four animals which received vaccine prepared from the blood alone and the four which received vaccine made from the organic tissues

As indicated in this Chart, the four head of cattle receiving the vaccine prepared from the blood were not protected As a matter of fact they showed no evidence of the slightest degree of immunity They developed rinderpest at the same time and in as severe a form as the controls On the other hand, the four animals which were vaccinated with vaccine prepared from the mixture of organs (lymph glands spleen liver, kidneys, testicles) all survived the rinderpest infection One of these latter four animals, however, had, at the time the test was started, a septic condition of the foot which later proved fatal It was thought in the beginning that the condition would not seriously interfere with the experiment and because the supply of susceptible cattle happened to be low at the time this animal was used It died however, of septicæmia before the test was completed

CHART 2.

Four of the ten head of cattle included in this chart each received three doses of vaccine prepared from blood alone, four received three doses of vaccine prepared from tissue alone and the remaining two served as controls Doses of vaccine given were 10, 15 and 15 c cs respectively, administered at weekly intervals

Animal No	Type vaccine	Date vaccination completed	Date infected	Amount of virus	RESULTS
6307	Blood	Oct 14, 1926	Oct 29, 1926	2 c cs	Developed acute rinderpest Would have died but killed to conserve tissues
6308	"	"	"	"	Developed acute rinderpest Would have died but killed to conserve tissues
6340	"	"	"	"	Developed acute rinderpest Would have died but killed to conserve tissues
6354	"	"	"	"	Developed acute rinderpest Would have died but killed to conserve tissues
6338	Tissue	"	"	"	Slight temperature reaction Survived
6341	"	"	"	"	No reaction whatever Survived
6346	"	"	"	"	This animal developed a septic condition of the foot and died before test complete
6349	"	"	"	"	No reaction Survived
6294		CONTROL	"	"	Developed acute rinderpest Would have died but killed to conserve tissues
6305		CONTROL	"	"	Developed acute rinderpest Would have died but killed to conserve tissues

The results obtained with the group of cattle vaccinated with the vaccine prepared from the mixture of blood and organs are shown in Chart 3

CHART 3

Eight of the ten head of cattle included in this chart each received three injections of vaccine prepared from a mixture of tissue and blood The doses were 10, 15 and 15 c cs respectively, administered at weekly intervals The last two animals were controls

Animal No	Date vaccination completed.	Date infected	Amount of virus	RESULTS
6296	Oct 14, 1926	Oct 27, 1926	2 c cs	No reaction whatever Survived
6319	"	"	"	Slight reaction Survived
6342	"	"	"	Slight reaction Survived.
6359	"	"	"	Slight reaction Survived
6361	"	"	"	Developed rinderpest in chronic form Died from same 24 days subsequent to administration virus
6310	"	"	"	Survived rinderpest infection but died later of unknown cause In poor condition from start
6362	"	"	"	Slight reaction Survived
6377	"	"	"	Survived infection without reaction
6293	CONTROL	"	"	Developed acute rinderpest Would have died but was killed to conserve tissues
6385	CONTROL	"	"	Developed acute rinderpest Would have died but was killed to conserve tissues

It is thus seen that seven of the eight vaccinated animals survived the infection with rinderpest virus One of the eight was not sufficiently protected and developed the disease This animal had the disease in a mild form in the beginning but it subsequently became chronic and finally resulted in death A second animal in this group survived the rinderpest infection but died later of an undetermined cause The two control animals promptly developed acute rinderpest in a severe form and were utilized for the production of vaccine

Following these results it was decided to discontinue the use of blood in the vaccine prepared by the Research Board

Tests to determine the Value of the various Organs for Vaccine Production

We prepared four lots of vaccine representing different tissues from a given group of animals infected for vaccine production One lot was made from the lymph glands another lot from the liver one from a mixture of kidneys and testicles and the fourth lot from spleen tissue

Ten head of cattle were employed for a test of these preparations Two animals were used for each type of vaccine and the remaining two served as controls The vaccinated animals received three 10 cubic centimeter doses of vaccine, a period of one week elapsing between doses The results of this test are given in Chart 4

CHART 4

Eight of the ten head of cattle included in this chart each received three doses of vaccine prepared from the various tissues indicated Each dose consisted of 10 c cs of vaccine administered at weekly intervals

Animal No	Tissues in vaccine	Date vaccination completed	Date infected	Amount of virus	RESULTS
6301	Lymph Glands	Nov 26 19 6	Dec 11 19 6	2 c cs	No reaction whatever Survived.
63_0					No reaction whatever Survived
6343	Liver				No reaction whatever Survived
6344					No reaction whatever Survived
6348	Kidneys and Testicles	"		"	No reaction whatever Survived
6365			"	"	No reaction whatever Survived
6371	Spleen		"	"	No reaction whatever Survived
6376				"	No reaction whatever Survived
6?98	CONTROL				Developed acute rinderpest Would have died but was killed to conserve tissues
6336	CONTROL		"		Developed acute rinderpest Would have died but was killed to conserve tissues.

All of the eight vaccinated animals survived the rinderpest infection without manifesting the slightest evidence of reaction The two animals used as controls promptly developed acute rinderpest and were destroyed for vaccine production

From this experiment it was evident that the tissues used were all of value for the production of vaccine

Test to determine whether or not one injection of vaccine will immunize

We gave two animals a single injection each of a lot of vaccine which had been made about 1½ months previously The dose given one was 25 cubic centimeters whereas the other received only 15 cubic centimeters Two weeks later these two animals together with a third used as a control, were each infected with 2 cubic centimeters of virulent rinderpest blood The results are shown in Chart 5

CHART 5

Two of the three head of cattle included in this chart received 15 and 25 c cs respectively, of a vaccine prepared from a mixture of tissue and blood Such amounts were administered in one dose The third animal served as a control

Animal No	Date vaccinated	Amount of vaccine	Date infected.	Amount of virus	RESULTS
6353	Oct 1, 1926	25 c cs	Oct 14 1926	2 c cs	Slight reaction Survived
6357	"	15 c cs	"	"	Developed acute rinderpest Would have died but was killed to conserve tissues
6363	CONTROL	"	"	"	Developed acute rinderpest Would have died but was killed to conserve tissues

A single injection of 25 cubic centimeters of vaccine protected against the artificial infection The animal receiving only 15 cubic centimeters developed the disease and died as a result thereof

It must be remembered that this lot of vaccine contained approximately 25 per cent blood which we later found to be of no value for immunizing purposes Had the vaccine been prepared without the blood, a single injection of a smaller amount would undoubtedly have sufficed

Keeping Qualities of Vaccine

In January 1927 we tested a lot of tissue vaccine which was three months old, on two head of cattle Each animal received three 10 cubic centimeter doses of

vaccine the injections being made at weekly intervals Two weeks subsequent to the last injection the two vaccinated animals together with two controls were each infected with 2 cubic centimeters of virulent rinderpest blood The results are pictured in Chart 6

CHART 6

Two of the four head of cattle included in this chart each received three injections of vaccine prepared from tissue alone The doses were 10 c cs Two controls included

Animal No	Date vaccination completed	Date infected	Amount of virus	RESULTS
6446	Jan 24 1927	Feb 8 1927	2 c cs	Slight temperature reaction Survived
6452				No reaction whatever Survived
6468	CONTROL			Developed acute rinderpest Would have died but was killed to conserve tissues
6483	CONTROL			Developed acute rinderpest Would have died but was killed to conserve tissues

The two vaccinated animals were fully protected by this vaccine The two controls developed acute rinderpest and were destroyed for vaccine production It is evident therefore that this vaccine will certainly retain its potency for at least three months and we have every reason to believe that it will remain potent for a much longer period We have on hand at present vaccine which is over a year old This will be tested in the near future for potency We had hoped to have this test completed before this meeting but because of a shortage of cattle for experimental purposes we had to defer it

It is of interest to note that Kakizaki Nakanishi and Oizumi in their work with a somewhat similar type of vaccine found it retained its potency for 2½ to 3½ years

Immunization of Carabaos

It is a well established fact that carabaos are considerably more susceptible to rinderpest than cattle It was therefore decided to test the immunizing value of our vaccine on carabaos For this purpose ten animals were employed Eight received 10 cubic centimeters of vaccine at a dose three doses being administered at weekly intervals The remaining two animals served as controls Two weeks subsequent to the last dose of vaccine each animal in the group was given 2 cubic

centimeters of virulent rinderpest blood subcutaneously. Chart 7 indicates the results obtained

CHART 7.

Eight of the ten carabaos included in this chart each received three injections of vaccine prepared from tissue alone The doses were 10 c cs each, administered at weekly intervals The last two animals served as controls

Animal No	Date vaccination completed.	Date infected	Amount of virus	RESULTS.
636	Jan 7, 1927	Jan 22, 1927	2 c cs	No reaction whatever Survived
637	"	"	"	No reaction whatever Survived
641	"	"	"	Very slight reaction. Survived
665	"		No virus	This animal had temperature at time others were infected so was not given virus Survived
667	"		"	This animal developed rinderpest before the others received virus artificially Died
668	"	Jan 22, 1927	2 c cs	No reaction whatever Survived
669	"	"	,	No reaction whatever Survived
670	"	,	"	No reaction whatever Survived
666	CONTROL	"	"	Developed acute rinderpest Died 12 days after receiving virus
675	CONTROL	"	"	Developed acute rinderpest Died 12 days after receiving virus

It will be noted that six of the vaccinated animals survived the infection with little or no reaction However, two of the vaccinated animals developed evidence of rinderpest (one only temperature evidence) before the virus was administered It is believed that they received the infection either by indirect contact or possibly through the vaccine in a particular bottle in which some virulent material may have existed between the stopper and neck of the bottle as up to this time we had paid no particular attention to this point One of these two animals died whereas the

one which showed only temperature evidence of infection survived That the temperature in the case of this latter animal was actually due to rinderpest was proved by the fact that the animal tolerated without reaction the subsequent administration of a large amount of virulent blood

Test of Vaccine containing 50 per cent physiological saline solution

As previously indicated after omitting the blood in the preparation of our vaccine the consistency of the mixture was such as to render injection difficult To overcome this we added an equal part of sterile physiological saline solution to the tissue mixture In order to prove that this addition of salt solution was not detrimental to the vaccine we subjected a lot of vaccine prepared in this manner to a test in which four head of cattle were employed Three animals each received three injections of vaccine at weekly intervals As the vaccine was diluted 50 per cent, the dose was increased to 20 cubic centimeters The fourth animal served as a control The results of this test are given in Chart 8

CHART 8

Three of the four head of cattle included in this chart each received three injections of vaccine prepared from a mixture of tissue and physiological saline solution The doses were 20 c cs each One animal control

Animal No	Date vaccination completed	Date infected	Amount of virus	Results
6186	March 1 1927	March 15 19 7	2 c cs	No reaction whatever Survived
6497				No reaction whatever Survived
6499				No reaction whatever Survived
6512	CONTROL			Developed acute rinderpest Would have died but was killed to conserve tissues

All three of the vaccinated animals survived the infection with virulent blood without reaction The control animal promptly developed acute rinderpest Since this test all of our vaccine has been prepared with physiological saline solution to facilitate injection

Dosage of Vaccine

With a view to obtaining information on proper dosage, a test was conducted in which eight head of cattle received three injections each of varying amounts of vaccine Two weeks subsequent to the last dose of vaccine these eight animals,

together with two controls, were infected in the usual way with rinderpest virus. The results are depicted in Chart 9.

Chart 9

Eight of the ten head of cattle included in this chart each received three injections of varying amounts of vaccine prepared from tissue. The doses were given at weekly intervals. Two animals served as controls.

Animal No	Dose of vaccine	Date vaccination completed	Date infected	Amount of virus	Results
6542	4 c cs	April 8, 1927	April 21, 1927	2 c cs	Moderate reaction. Survived
6545	4 c cs	,,	,,	,,	Slight reaction. Survived
6537	8 c cs	,,	,,	,,	No reaction whatever. Survived
6538	8 c cs	,,	,,	,,	Developed no evidence of rinderpest but died of acute indigestion at later date
6549	12 c cs	,,	,,	,,	Slight temperature reaction. Survived
6550	12 c cs	,,	,,	,,	This animal was injured before vaccination was completed. It survived rinderpest but died result of injuries later
6521	16 c cs	,,	,,	,,	Moderate reaction. Survived
6522	16 c cs	,,	,,	,,	No reaction whatever. Survived
6606	.	Control	,,	,,	Developed acute rinderpest. Would have died but killed to conserve tissues
6614	.	Control	,,	,,	Developed acute rinderpest. Would have died but killed to conserve tissues

The results of this test were somewhat disappointing in that the smallest dose administered was sufficient to protect and no information on the minimum amount was obtained None of the eight vaccinated animals developed rinderpest although two of the group died later from other causes not incident to the vaccination or rinderpest infection The two control animals promptly developed acute rinderpest

Number of doses of Vaccine necessary to immunize Carabaos

It is obvious, of course, that rinderpest control work would be greatly facilitated if the number of doses of vaccine for immunization purposes could be reduced As already pointed out we determined that cattle could be immunized with a single dose of vaccine Carabaos being more susceptible than cattle the question arose as to whether they too could be satisfactorily protected with less than three doses of vaccine

A test was conducted in which five carabaos were used Two received one injection each of vaccine, two were given two doses each and the fifth animal served as a control The results are shown in Chart 10

CHART 10

Two of the five head of carabaos included in this chart received two injections each of 60 c cs of a mixture of tissue vaccine and saline solution Two received one injection each of 60 c cs of the mixture The fifth animal served as a control

Animal No	Doses of vaccine	Date vaccination completed.	Date infected	Amount of virus	RESULTS
723	1	July 30, 1927	Aug 20, 1927	2 c cs	Developed rinderpest and died as result 12 days subsequent to infection
725	1	"	"	"	No reaction Survived
696	2		"	"	No reaction Survived
724	2	"	"	"	Moderate reaction Survived
6644		CONTROL	"	"	Developed acute rinderpest and would have died but killed to conserve tissues

As indicated in the Chart one of the two animals which received single doses of vaccine failed to resist the infection with virulent blood Both of the animals which received two doses of vaccine were fully protected

It was thus demonstrated that carabaos can be immunized against a severe artificial infection with two doses of vaccine whereas with only a single dose of our combined tissue vaccine the immunity conferred is not sufficient to protect all animals against artificial infection

In this test the dose of vaccine was large (60 c cs) The employment of such large doses of vaccine involves considerable technical difficulty and, further causes considerable tissue irritation at the point of injection However since this test Dr E A Rodier who is in charge of the rinderpest vaccine production for the Insular Bureau of Agriculture in a very clear cut experiment fully protected carabaos with single injections of 10 and 15 cubic centimeters of vaccine made according to our method but from the lymph gland tissue alone It thus appears that if vaccine is made only from the tissue of highest potency single doses can be employed for the immunization of carabaos There is an economic question involved however, as the lymph glands constitute only a relatively small percentage of the various tissues proven of value for vaccine and consideration must be given this factor

We contemplate further work with dosage of our rinderpest vaccine

Conclusions

(1) A highly efficacious vaccine against rinderpest can be prepared from a suspension of finely ground tissues (lymph glands spleen liver kidneys testicles) from animals killed in the acute stages of rinderpest

(2) We have demonstrated that the rinderpest virus in such vaccine can be promptly killed without injuring the product by the addition of 0 75 per cent chloroform Such vaccine can be used immediately after preparation and possesses good keeping qualities

(3) Vaccine prepared from blood alone possesses no value whatever as an immunizing agent Thus as the rinderpest virus is present in the blood in large amounts during the acute stages of the disease it is apparent that the active principle of the tissue vaccine is not merely virus killed by chloroform Whatever it is it is not present in demonstrable amounts in the circulating blood but only in certain of the other tissues This suggests the possibility that the immunizing principle is either some by product of the reaction between tissue and virus or the rinderpest virus which has been changed in some particular way by the activity of the tissue

(4) Tests of this vaccine on a large number of cattle and carabaos used in the various experiments have fully established its worth

(5) Three 20 cubic centimeter doses of vaccine with an interval of one week between doses affords a solid immunity against severe infection

(6) For the vaccination of cattle our experimental evidence indicates that for practical purposes the number of doses can certainly be reduced to two of 20 or 25

cubic centimeters and possibly with reasonable safety to one dose of 25 cubic centimeters

(7) For the vaccination of carabaos two or even three doses are advisable where the vaccine is prepared from a mixture of the various tissues If the vaccine for carabaos is prepared only from the tissues which are apparently of highest potency (lymph glands, spleen) then a single injection of 15 to 20 cubic centimeters is probably sufficient

REFERENCES

(1) KOCH (1897) — *Centralb f Bakt Parasit u Infektionsk* Vol XXI, p 526

(2) KOHLSTOCK (1897) — Arbeiten aus dem Kaiserlich Gesundheit, Vol XXII, p 787

(3) KOLLE (1898) — *Zeit f Hyg u Infektionsk* Vol XXVI p 45

(4) THEILER (1898) — *Deutsche tierarzliche Wochenschrift,* p 205

(5) TURNER (1898) — *Zeit f Hyg u Infektionsk*, Vol XXIX p 309

(6) EDINGTON (1926) — Quoted from Huytra and Marek Special Pathology and Therapeutics of the Diseases of Domestic Animals Eng trans, 3rd Ed Alexander Eger Co Chicago Ill

(7) KOHLSTOCK (1897) — *Deutsche tierarzliche Wochenschrift* Vol XXII, p 787

(8) SEMMER (1893) — *Berliner tierarzliche Wochenschrift* p 590

(9) KOLLE and TURNER (1926) — Quoted from Huytra and Marek Special Pathology and Therapeutics of the Diseases of Domestic Animals Eng trans 3rd Ed Alexander Eger Co Chicago Ill

(10) BOYNTON — Official Records of the Insular Bureau of Agriculture, Manila P I

(11) KAKIZAKI *et al* (1925, 1927) — Third and Fourth Reports of the Government Institute for Veterinary Research Fusan, Chosen, Japan December and June

DISCUSSION

Mr J T Edwards (United Provinces) I have listened with interest to Col Vedder s reading of Major Kelser's paper, for the reason that we have striven for some years at Muktesar to ascertain whether infective products obtained from the tissues of animals suffering from rinderpest and treated in various ways so as to render them innocuous, are capable of setting up a vaccinating effect upon inoculation into susceptible animals Hitherto our attempts, when conducted upon a sufficiently large number of experimental animals (Himalayan hill cattle), have proved fruitless We have treated the vaccinated tissues with toluol, glycerin, chloroform and other agents Col Vedder now assures me that the percentage of chloroform (0 75) recommended by Major Kelser is not believed to have any special virtue in itself He further mentions that, in the Philippines, the local carabaos (buffaloes) are far more susceptible than the cattle (which are apparently of Indian importation) whereas the cattle are protected by a single injection of the vaccine, the buffaloes require three or more injections (of about 25 c cs each) Now, in India, we do not regard the buffalo as a highly susceptible animal although the susceptibility of the species, as compared with that of the cattle, is a fairly uniform one Judged by the quantity of anti rinderpest serum required for their protection, buffaloes

are three or more times more resistant to infection than the hill cattle used for rinderpest researches at Muktesar, while some of the plains cattle may be about three times as resistant as the buffaloes It is not unlikely therefore that the Philippine authorities have been operating upon relatively resistant cattle, comparable in their resistance to the least susceptible plains cattle of India and hence material containing the inert rinderpest virus may provoke upon inoculation into them a mild response followed by the slight additional immunity which would give them for a period afterwards complete resistance to natural infection It seems that the numbers of animals observed by Major Kelser were small

Mr K Hewlett (Bombay) What length of time does the immunity last against rinderpest ?

Mr T F Quirke (Punjab) Wanted to know to what extent this improved vaccine for immunization against rinderpest had been brought into practical use in the Philippines and to what extent it had displaced the more common serum inoculation method in dealing with rinderpest He was also interested to know what the owner of stock in the Philippines thought of this method and to what extent they show willingness to employ this method of vaccination for the protection of their cattle

Lieut Col Edward B Vedder (Philippine Islands) replied 0 75 per cent chloroform was simply selected as the smallest amount that would kill the vaccine

This method is not now in general use in the Philippines because Boynton's vaccine has been in use for a number of years However, this investigation was undertaken at the request of the Department of Agriculture and this department is now installing the apparatus for the production of Kelser's vaccine on a large scale

We do not know how long immunity lasts after the use of this vaccine, but it certainly does protect carabaos which are more susceptible than cattle, from rinderpest at the termination of vaccination since they are then resistant to injections of living virus

THE TREATMENT OF CANINE PIROPLASMOSIS

BY

MAJOR R F STIRLING F R C V S D V S M F Z S I V S,
*Second Superintendent Civil Veterinary Department Central Provinces,
at present Officiating Pathologist Imperial Institute
of Veterinary Research Muktesar*

INTRODUCTION

DURING the past seven years the writer has had several opportunities to study the question of the treatment of canine piroplasmosis as piroplasmosis generally is the most widespread disease affecting animals in the Central Provinces There are at least two different species of piroplasm parasites which affect dogs in India These are —

(a) *Piroplasma (Babesia) canis* the cause of the so called malignant jaundice Infection with this piroplasm has long been known to yield in a remarkable manner to treatment with the aniline dye trypan blue

(b) *Piroplasma gibsoni* —The affection caused by this much smaller piroplasm differs clinically from that caused by *P canis* in that it is usually more prolonged in its effects and the symptoms are generally those of a progressive anæmia with a frequent tendency to relapses at prolonged intervals It is known now to be surprisingly common in many parts of India It does not respond, it would seem, to treatment with trypan blue but good results have been obtained by repeated treatment with certain arsenical preparations, notably so far as the writer's experience goes, tryparsamide

THE USE OF TRYPAN BLUE IN *P canis*

(1) *Preparation of the solution* —The brand of trypan blue recommended is that prepared by the German firm Merck Darmstadt The drug supplied by this firm is eminently superior in properties to the products the writer has been able to secure elsewhere In the years 1921 and 1922, supplies were obtained from a British firm for trial and they were found to be less soluble than the German product and frequently became gelatinous in solution on cooling The drug is made up in two per cent solution in water The powder is weighed out into a mortar, and finely triturated with a small amount of cold water into a paste, more water is then gradually added until the dye enters completely into solution,

which is then made up to the desired bulk The solution is passed through ordinary filter paper, and the filtrate is transferred to a wide mouthed flask, which is then placed in an autoclave maintained at a temperature of 120° C for 20 minutes When the solution in the flask has been cooled it is distributed into sterile bottles which are then carefully sealed, and the material used as required

(2) *Dose* —The dose for the dog is from 5 to 6 c cs This dose may appear to be rather low but as the village dogs are very frequently much sunk in condition it has been considered prudent to recommend for adoption a dose rate somewhat lower than what is sometimes recommended Larger doses were sometimes followed by symptoms of dyspnœa and temporary collapse and although these symptoms passed off without any untoward incident to the animal itself they caused alarm in the mind of the owner, who might easily be led to refuse further treatment of his animal after the experience The smaller dose was therefore deemed adequate to be repeated after an interval of a few days to a week, at the most

(3) *Mode of administration* —When the drug was first issued in the Central Provinces it was administered subcutaneously , later, however, the subordinate staff were trained in the proper method of administering it intravenously into the external saphena vein

While the possibilities of the drug were being explored during the first year, some interesting practical information was obtained upon the subcutaneous injection of trypan blue into dogs Wallis Hoare(1) recites the African experience that injection in this way is attended with untoward consequences in dogs as there was a great tendency to abscess formation if aseptic precautions were not taken ' The experience we gained in the course of our work was similar, so long as the injections were made into the inner flat surface of the thigh The proneness to abscess formation at this site was thought to be associated with the likelihood of the animals conveying infection from their anal and genital region to the seat of injection by repeated licking of one part and then the other, and so various other parts of the body surface were tried for inoculation It is found that abscess formation became extremely rare when the injections were given either subcutaneously on the side of the neck , or intramuscularly, by deep injection into the gluteal muscles In fact when abscess formation did occur it was almost invariably traced to lack of necessary precautions on the part of the operator

Results

The results obtained by the author in the treatment of *P canis* in India by trypan blue confirmed those recorded by veterinary surgeons elsewhere in the world The drug is a specific one for this form of piroplasmosis and all that can be claimed by the writer is that he has slightly modified the mode of preparation of the solution

The Use of Trypapsamide in *P gibsoni*

The author's experience regarding the efficacy of this drug does not agree with that of General Symons(2)

(1) *Mode of preparation of the solution* —A solution of normal saline is first subjected to sterilization in the autoclave maintained at 120° C for twenty minutes When the solution has cooled, the drug is added to it at the rate of 0 85 gramme per 5 c cs The material is then bottled in the required quantities and the bottle hermetically sealed Particular care is taken that all containers, flasks and bottles are sterile and when making large quantities of the solution it is recommended to examine the purity of the preparation in various stages by ordinary laboratory tests

The following quantities are given for easy reference —

Water—630 c cs (30 c cs for evaporation)

NaCl—4 8 grms

Autoclave

(If necessary, remove the amount in excess of 600 c cs)

When cool add 102 grms tryparsamide

(2) *Dose* —The dose for a cocker spaniel weighing about 25 lbs is 5 c cs , that is 0 85 grm of the drug For hounds 10 c cs or 1 7 grms are used, whilst for dogs of 10 to 12 lbs in weight, 2 5 c cs or 0 42 grm seems to be sufficient, and for unweaned pups of any breed 0 5 to 1 c c gives good results The writer's experience goes to show that the injections must be repeated and the best results were obtained by giving five injections at five day intervals It was found that in exceptional cases a still larger number of injections was necessary making eight in all The results so far obtained go to prove that some dogs show no parasites after the third injection, but it is advisable to continue the treatment until at least five injections have been given It will be seen therefore, that it takes on an average 20 days to complete the course and occasionally 35 days When an attempt was made to shorten the course by increasing the dose of the drug it was found that certain untoward symptoms arose manifested especially in the form of lack of co ordination in the motor system , this was a temporary condition but quite sufficient to arouse slight alarm in the mind of an owner of valuable dogs

(3) *Mode of administration* —The drug is given preferably into the external saphena vein, but it is administered with the greatest safety subcutaneously into the side of the neck and this is to be regarded as a point of importance where the treatment has to be entrusted to laymen One definite record has been obtained which shows that out of 650 subcutaneous injections all done by laymen, not one was followed by the occurrence of abscess formation

Results

The results obtained from the employment of the drug in the manner indicated above may be declared to be satisfactory However, in clinical veterinary work in

India it is for many reasons, difficult to keep accurate record of cases and the brief report which now follows relative to an outbreak of *P gibsoni* infection in the United Provinces attains special significance in that the cases alluded to therein were kept under observation from practically the commencement of infection until the termination of treatment

The dogs affected numbered 126 and were those of kennels belonging to one owner The varieties comprised spaniels hounds, retrievers pekinese and poms (a few) and some other small breeds The majority of these animals were imported from England or were the progeny of imported dogs For about two years losses amounting to over 25 per cent of the kennels occurred each year, and the deaths had been attributed to various causes On examination of the animals and of blood smears 78 per cent of them showed the presence of *P gibsoni* and 11 had a mixed infection with *P canis* and *P gibsoni* All the 126 animals were subjected to treatment with tryparsamide After three injections examination of blood smears showed that the number showing piroplasms had been reduced to 18 per cent and after five injections only four dogs revealed sparse *P gibsoni* These four were given three more injections and their blood was then found clear During the season under review only one death occurred and this was in the case of a spaniel which died a day after the first injection

Summary

(1) Trypan blue is a specific for piroplasmosis caused by *P canis*

(2) The formation of abscesses following the use of trypan blue administered subcutaneously is of rare occurrence if the solution employed for injection is duly sterilized ordinary antiseptic precautions are taken and a suitable site is chosen for injection

(3) Good results have been obtained by the use of tryparsamide in repeated doses in piroplasmosis due to the *P gibsoni*

(4) Tryparsamide will also rid the blood of *P canis*

(5) Tryparsamide can be safely used subcutaneously

Note

Some of the data given concerning trypan blue are included in an article already submitted for publication in an English journal The writer wishes to tender his sincere thanks to Mr J R Haddow B SC M R C V S D V S M for many suggestions regarding the administration of tryparsamide and also to express his gratitude to Dr J T Edwards Director of the Imperial Institute of Veterinary Research Muktesar, for his valuable help in the preparation of this paper

References

(1) Wallis Hoare System of Veterinary Medicine Vol I p 103.

(2) Symons, T H (1926) *Ind Jour Med Res* Oct, p 93

DISCUSSION

Mr J T Edwards (United Provinces) In presenting this paper on the treatment of canine piroplasmosis to the meeting I may add that I do not think my colleague Major Stirling has it in his mind to claim any priority in regard to the method of treatment of *Piroplasma gibsoni* infection by means of arsenical preparations Treatment with salvarsan was recommended after trial by the early workers upon this infection, who first recognized and described it as occurring in Madras It has been the natural outcome of circumstances that one of the newer arsenical compounds, tryparsamide should have been tried, for it possesses at least one property which makes it superior for use upon dogs to the older preparations, in that it does not induce abscess formation on subcutaneous injection The special claims to recognition of the work now recorded by Major Stirling are that it was carried out on a large establishment comprising valuable dogs which were suffering for the most part rather severely from the effects of *P gibsoni* infection, and that he was able to continue careful observation upon the dogs until the end of the treatment so that its utility as recommended may be accepted with a high degree of assurance This kind of piroplasmosis or as it is commonly known, tick fever is extremely common and widespread in Northern India and it is of interest to note that it may be frequently complicated with *P canis* infection In the past the real nature of the disease has often remained undiagnosed for on account of the minute size of the piroplasms they may readily pass undetected in blood films examined by individuals who are inexperienced or have in mind the morphology of the larger piroplasm *P canis* in the course of search In an outbreak one is often struck by the large numbers of the *gibsoni* parasites infecting the blood cells even in dogs that show merely slight or inappreciable symptoms

Mr V Krishnamurti Ayyar (Madras) Major Stirling says ' The drug is made up in 2 per cent solution in water The solution is filtered autoclaved for 20 minutes at a temperature of 120° C cooled and distributed into sterile bottles ' Trypan blue is an organic synthetic compound and I would like to know whether its composition would not be affected by exposing the solution of the dye in an autoclave to a temperature of 120° C for 20 minutes Nuttall has stated that the dye should be dissolved in sterile water at blood heat, probably owing to the possibility of the dye breaking up if exposed to a high temperature I may also add that even for the purposes of media for growing bacteria various sugars are not autoclaved for the above reason and I fail to see the necessity of autoclaving the trypan-blue solution to 120° C for 20 minutes If it is considered that by autoclaving the solution efficient sterilization is obtained, then there appears to be no force in transferring the solution after being autoclaved into sterile bottles, for there is the same fear of contamination creeping in during the transfer of the solution into the bottles

Major Stirling attributes the proneness of abscess formation to the likelihood of the animals conveying infection from their anal and genital regions to the seat of injection by repeated licking of one part and then the other If it were so, injection of any material other than the trypan blue at the region should produce similar abscesses but it is not the case I may add that when the injection is finished there appears to be no possibility of any infection creeping through the seat of inoculation and, if it is so, any other parts of the body are as susceptible as the thigh region for they are exposed to

to the same risk of contamination when dogs lie on the floor and rub the parts against anything I have done some tests with a solution of trypan-blue and it appears to possess some germicidal effect

The term abscess formation is not correct since the changes set up at the seat of injection are more of the nature of a slough This condition is brought about by the undissolved particles of the dye setting up irritation and producing the slough This frequently occurs in the thigh because the skin of the part is very thin as compared with that of any other region and the chances of sloughing are therefore greater In my experience, so long as the solutions are made up from 0 5 to 1 per cent in sterile water there is little likelihood of any slough being set up

In this connection, I may add that in field work when we are conducting anti rinderpest inoculations in big herds, we do not sterilize the syringe every time and no cases of abscess formation have occurred

I would also like to be enlightened with regard to the preparation of tryparsamide solution Major Stirling says ' It is recommended to examine the purity of the preparation in various stages by ordinary laboratory tests ' I would like to know what laboratory tests he advocates in the different stages, i e, between the preparation of the solution and the injection which follows immediately

Mr F Ware, I V S (Madras) Some three years ago the use of tryparsamide and novarsenobilion (N A B) was introduced for the treatment of *P gibsoni* infection in imported fox hounds in Madras, and although both were successful, the general impression left on the users was that N A B was the safer drug of the two This latter drug was therefore solely used during the last cold season, in which nine confirmed cases were dealt with and all recovered

0 15 gramme the full human dose, was used as the initial dose, followed up with 0 3 gramme as often as necessary One case received five doses in 22 days and showed no ill effects from the drug This drug needs to be given intravenously with great care, in which matter tryparsamide shows a very great advantage

Col A J Williams, R A V C (B India) In my experience abscess formation invariably follows one injection of 1 c c of a 1 per cent solution of trypan blue to a pound body weight when injected into the inner surface of the thigh no matter how thorough the precautionary measures but it rarely occurs when small and repeated doses are used It therefore appears that the cause of this condition is more likely due to the irritating nature of the drug than infection of the seat of injection by the animal licking the part

Rai Sahib Debal ar Dey (Bengal) Abscess formation after subcutaneous injections of anti rinderpest serum is perhaps due to the ½ per cent carbolic solution being added to the anti rinderpest serum at Muktesar Laboratory before the brew of serum is bottled

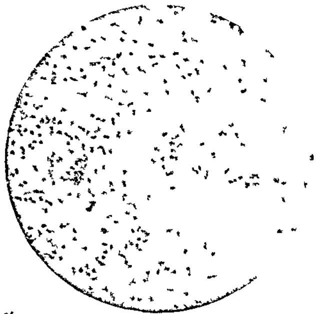
Abscess formations after subcutaneous injections of trypan blue —Quite recently while attending the clinics of the Royal Veterinary Colleges of Edinburgh and London, I saw subcutaneous injections of this drug properly diluted (fresh), well mixed and used warm, upon horses, dogs and cattle, without a single case of abscess formation I submit, much depends upon the cellular resistance of the case, proper brand and age of the trypan blue and sterility of the sites of injections

Mr J T Edwards (United Provinces) In replying to the questions raised by the speakers I may say that I am unable to judge exactly what form of answer to them my colleague Major Stirling would be inclined to give However in the administration of trypan blue to cattle and dogs for the treatment of piroplasmosis he has had a very large experience in the Central Provinces and the precautions described by him in the preparation of solutions of this drug would be based upon this experience Mr Krishnamurti wondered whether autoclaving of the solutions is necessary Some time ago it was generally recommended that the solutions should be freshly made by boiling the powdered dye in one per cent solution in water filtering through paper and injecting it as soon as it had cooled down to about body temperature This procedure was recommended because solutions of the trypan blue then obtainable had a tendency to gelatinize on cooling Major Stirling finds that the trypan blue obtainable from the German firm Merck does not change in this way and so solutions of it can be safely autoclaved as a precautionary measure and then stored for some time before issue

It is curious that in Major Stirling s observations the repeated treatment with tryparsamide cleared up the *P canis* as well as the *P gibsoni* infection I had in mind at first that a combination of tryparsamide and trypan blue would be necessary for the mixed infections but it seems that the *canis* infection also yields to the continued treatment with tryparsamide

It is very difficult to explain the peculiar susceptibility of the canine species to abscess formation after the subcutaneous injection of trypan blue it seems difficult to accept entirely the explanation that it is caused by licking of the seat of injection

Further work needs to be done in calculating the dose that is just necessary to bring about the disappearance of the piroplasms at each injection largely to economize in the cost of treatment There is no reason why the drug should not be administered as often as once daily or once every two days if treatment at more frequent intervals were desirable for in my researches on the treatment of surra in horses commencing as far back as 1922 it was found that the drug was excreted so quickly from the system that a therapeutic dose given intravenously could not prevent the establishment of trypanosome infection even when the drug was given concurrently with the infected material



Γ 1

(1 c oj botograj h of a sta ned smear Ze ss Apoc ro n t mm K upen (Enla re l 2 t mes of th o nal

r 6

r

Fou day cultu e on ral l t ae m l t ea l in l v l l m as ng ale t (n—O t/ n l n t

I g a

(H Ha f (n t Sta n ng an l M f l lgy f tl n l (nta a l f a f Mil)

The micro organisms are stained intensively bichromatic with this method The organisms are round oval or cocco bacillary in shape and measure circa 0 2—0 4 μ in diameter and they are arranged in mono or diplo coccoid tetragenous and sarcina like forms, or the form of short chains but originally they have no special arrangement Thus they are mono-coccoid In old cultures the evolution of the form to the filament vibrio spirillar or into S form can be observed but the typical form of the virus should be mono coccoid or cocco bacillus Hence we cannot agre in designating the virus is micromyces (Frosch) or *Asterococcus mycoides* (Borrel) the names given to the virus on account of its pleomorphism which has been assumed to be the special characteristic of the virus though it might have been an accidental concomitance of various foreign bodies which caused the confusion in the observations of previous investigators

DISCUSSION

Mr J T Edwards (United Provinces) In the course of researches into the virus of rinderpest I have endeavoured to obtain information upon the factors which may be necessary for the successful cultivation by studies upon the virus of contagious bovine pleuro pneumonia and I have failed to be convinced that the designation which has become current in the literature *Asterococcus* fairly represents the morphology of the organisms The figures given by Bordet of the organism many years ago and which are reproduced in standard works of reference (Koll and Wassermann Hutyra and Marek) depict a minute organism which is definitely vibrionic or lepto spirillary in form and the aberrant appearances presented by some of the structures might be explained by the distortion produced in these delicate bodies after subjection to the treatment for microscopic examination As ordinarily seen on a glass slide after staining especially after the use of staining processes in which a mordanting fluid is employed the bodies are coarsely occoid but this appearance is almost certainly attributable to the brutality of the echnique Great care has also to be taken not to confuse the coccoid or protein particles ound in the albuminous fluid in which the virus most readily grows with the actual ganisms

In their ætiological researches upon cancer recently Gye and Barnard made use of s virus to ascertain the reliability of their method of determining the presence of an ramicroscopic virus by ultraviolet microphotography Barnard in this manner red curiously shaped bodies that underwent a peculiar evolution I am not convinced such structures were not the result of a reaction set up in the fluid by the growth he virus the appearances seen in cultures on examination under the dark ground oscope are certainly confusing and necessitate frequent reference to control culated medium

PLATE XXXI

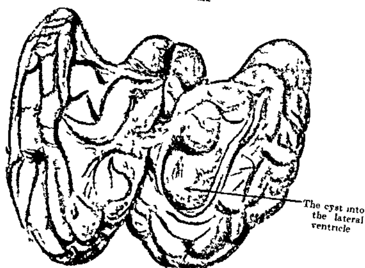


Fig 1

Size Goat
1 9 26



Fig 2.

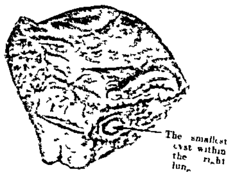


Fig 3.



Fig 4

other inside the right thigh Both were operated upon and the cysts remov
intact When examined under microscope the heads presented large and sm
hooks, the latter having bifid guards Two dogs were experimentally fed wi
portions of the cysts and they began to pass mature segments of a *Tænia serial*
in their stools, within six weeks from the date of feeding the goat showe
symptoms of colicky pain and brain trouble (meningitis etc) and died three
months after the date of operation

Autopsy on the goat revealed that in one of the lateral ventricles of the brain was a fairly good sized cyst (Plate XXXI, fig 1)

A second one was found resting on the lung and hanging from the pulmonary pleura (Plate XXXI fig 2), the smallest cyst was within the right lung (Plate XXXI, fig 3) and, lastly the largest cyst was attached to the uterus (Plate XXXII, fig 4)

Four more dogs were fed with different parts of the cysts and all the four dogs began to pass segments of a *Tænia serialis* The dogs were destroyed and, when examined, their stomachs were found packed up with *Tænia serialis*

Now let me refer to the differential points between the well known helminths, *Multiceps multiceps* and *Multiceps gaigeri*

Multiceps multiceps	*Multiceps gaigeri*
(1) Found generally in sheep and goats and less commonly in calf	(1) Found in hare rabbit and squirrel and goat
(2) Found mostly in the brain tissue	(2) Generally found in the subcutaneous tissue
(3) Found as a rule single	(3) The cyst forms daughter cysts
(4) Smaller hooklet, has only a single entire guard	(4) Smaller hooklet has a bifid guard.

In India, so far as I am aware Gaiger was the first to report the presence of *Multiceps serialis* in the subcutaneous tissue of goats from the Punjab Veterinary College But I have found the cystic stage of the parasite not only in the subcutaneous tissue but also in the viscera and the brain, in the two cases referred to above

REFERENCES

Nathusius — 'Neumann's Parasites, p 547

…ichler — *Ibid* p 547

Ransom — 'The Gid Parasite (*Cœnurus cerebralis*) its presence in American sheep,' p 11

…aiger (1907) — *Jour Trop Vet Sci*, Vol II, No 3, pp 316—321

…y (1909) — *Ibid* Vol IV, No 4, pp 556—560

RHINOSPORIDIOSIS IN CATTLE

A CASE RECORDED IN A BULLOCK

BY

V KRISHNAMURTI AYYAR, G M V C I V S,

Professor of Pathology and Bacteriology, Madras Veterinary College

THE earliest recorded occurrence of rhinosporidiosis in man dates back to the year 1900 when Seeber discovered the parasite in growths from the nasal cavities of two men living in Argentina

In the year 1903 O Kinealy was first to describe in India a similar parasite in the material which he had obtained from the nasal cavity of one of his patients in Calcutta, and Tantham and Minchen, to whom the material was sent published an account of the developmental sequence of the organism which they named as *Rhinosporidium kinealyi* and which has now been redesignated as *Rhinosporidium seeberi* owing to the priority of the discovery of the parasite by Seeber Since then more cases of rhinosporidiosis have been recorded in man in India by various workers Beattie Elliot Ingram Kirkpatrick Tirumurti and R F Wright by Castellani and Chalmers and Chelliah in Ceylon and J Wright in the United States Most of the cases recorded in India were from the West Coast, Cochin State Madras Trichinopoly Dindigul and Tinnevelly and in the great majority of such cases the lesions which were in the nature of polypi were found in nasal cavities and naso pharynx while in a few others they were present on the uvula conjunctiva lachrymal sac penis and the ear

In the year 1923 in a paper entitled ' A Case of Rhinosporidiosis ' Ashworth and Turner have described the affection in its various aspects

As regards its prevalence in animals Zschokke was first to record its occurrence in the nasal tumour in a horse in South Africa It is reported that the tumour was round and about the size of an egg reddish and hard with a rough outer surface and that inside were found cysts which were filled with a parasite allied to *Rhinosporidium seeberi* which he termed *Rhinosporidium equi*

Last year, two more cases were recorded by J Quinlan and G de Kock in the same country It is reported that one of these mules showed a number of pinkish tumour like growths with irregular masses in the nasal mucous membrane the smallest measuring about 2 mm in diameter and 1 mm in height while the larger which appeared to be formed by the coalescence of smaller growths measured two centimetres in diameter and 1 5 cm in height and that in these growths cysts

containing spore morulæ of Rhinosporidium were present The other mule it is stated, exhibited signs of dyspnœa when trotting and made a snoring sound during respiration There was an intermittent discharge from the left nostril Surgical interference revealed the presence of a large granulation like mass practically filling the left nostril Higher up in the fossa the membrane was studded with growths Histologically the growths resembled those above described in the mule

Beyond these cases, its occurrence has not been reported either in the equines or in any other animals in any other country even in India where cases in man have been recorded

During the early part of the year 1922 I came to know of the existence of a disease condition among cattle in the Madras Presidency known as nasal granuloma, or popularly 'snoring disease,' in which it was reported that growths were found in the nasal cavity obstructing the nasal passage and which from the nature of the prevalence appeared to be infective in origin The possibility of the affection being due to Rhinosporidium suggested itself to me and I made arrangements with the help of the Principal, Madras Veterinary College and the Chief Superintendent, Civil Veterinary Department Madras, to obtain materials from affected cases In most of the materials that were sent to me and in the few that I personally collected the growths appeared of the nature of chronic granulomatous formations and histologically in the follicles of such formations were found granules which were very similar in appearance and texture to the 'ray fungus' of actinomycosis but differing from it in being relatively acid and alcohol fast The results of my investigations were published first in the *Madras Veterinary Journal* in July 1922 and later as one of the *Memoirs of the Department of Agriculture* in August 1923 entitled 'Nasal Granuloma in Cattle'

Towards the middle of February of this year material was received from Veterinary Assistant Surgeon S Ramaswamy in charge of the Veterinary Hospital, Tanjore, purported to have been removed from the nasal cavity of a bullock with the following history sheet —

History sheet of growth

District	Tanjore
Taluk ..	Tanjore
Village ..	Tanjore
Animal sex and class .	Bullock, male
General condition	Good
Age	Aged
Alive or dead ..	Alive
Temperature if alive .	Normal.
Seat from which the growth was taken ..	Nasal chamber
Date of removal	17 2 1927
Disease suspected	Nasal Granuloma
Special symptoms	This was obstructing the nasal cavity making the animal breathe hard and snore Scissored off as the growth was within reach

The material on naked eye examination presented quite a distinct feature from those which are usually met with in cases of nasal granuloma The specimen showed a peculiar cauliflower like appearance and was studded with a number of irregular but smooth granular growths which were easily friable and which on close *inspection revealed minute pale spots* Towards the centre of the growths there was easily visible a flat fibrous band of connective tissue like the midrib of a leaf which was seen to branch in various directions Owing to the specimen being received preserved in formalin the true colour of the fresh specimen could not be stated

On histological examination there were seen a number of cysts or sporangia which were quite similar to those described in human rhinosporidiosis

The appearance presented by the epithelial lining was very striking The epithelial layer was found thrown into irregular folds some of which appeared more or less like flask shaped invaginations

The sub epithelial connective tissue was very vascular with numerous young and newly developed vessels with œdematous infiltration Under the surface epithelium there appeared as it were a channel in which sporangia in different stages were abundant In some areas there was thinning of the surface epithelium due to the pressure of the fully developed sporangia while in others rupture of the epithelium and of the sporangia with the escape of spores was noticed

Each of the cysts or sporangia was found to possess a wall varying *in thickness* In some the wall was found considerably thickened while in others especially in fully developed sporangia it appeared to be progressively decreasing in thickness

The spores contained in the sporangia were in different stages These were either fully formed spores in the centre and small ones at the periphery or *fully* formed spores at one pole and small cells at the other with intermediate *stages in* the intervening areas The fully formed spore contained bodies which have been considered by Ashworth and Turner to be *refrangent spherules and by other* workers to be sporules or sporozoites

I tried to obtain fresh material from this animal for further study but the Veterinary Assistant Surgeon reported that the animal was sold away in the interior parts of the district and that it was not possible to send *more material* for examination *However from what has been recorded above and the* photographs appended to this paper it will be seen that *the situation of the* polypus its gross appearance histology structu[illegible] the sporangia and spores are all quite identical with those d[illegible] an r[illegible] *ridiosis* Rao Sahib T S Tirumurti to whom I sh[illegible] *of the* section also agrees with my view

The discovery of the parasite in cattle [illegible] man rhinosporidiosis have been reported rar[illegible] the parasite described in the paper is the sa[illegible] closely allied species If it is the form[illegible] diseases common between man and

between the two It would also appear that the cause of nasal granuloma in cattle is not of one ætiological entity but of two, namely, an organism akin to *Actinomycosis bovis* which is present in most of the cases and the other a Rhinosporidium and that although the clinical symptoms due to both the organisms appear to be more or less the same, the characters of the growth, the histological changes produced by each of the parasites, are definitely different

REFFRENCES

ASHWORTH, J H, and TURNER A LOGAN (1923) — A Case of Rhinosporidiosis *Jour of Laryngology and Otology*, June

KRISHNAMURTI AYYAR, V (1925) — Nasal Granuloma in Cattle, *Memoirs of the Department of Agriculture in India* Veterinary Series, Vol III No 6

QUINLAN, J and DE KOCK, G (1926) — Two Cases of Rhinosporidiosis in Equines *South African Journal of Science* December Vol XXIII, pp 589—594 Abstracted in *Trop Vet Bull*, September 1, 1927

TIRUMURTI T S (1914) — *Rhinosporidium kinealyi* *The Practitioner* pp 704—719

ZSCHOKKE, F (1913) — Ein Rhinosporidium lesim Pferd *Schweizer Arch f Tierheilkunde*, December Vol LV No 12 pp 641—650 Abstracted in *Trop Dis Bull*, Vol VI p 202

ADDITIONAL NOTE

Since sending the above paper to the General Organizing Secretary of the Congress, two more cases have been recorded in cattle by me The history of these cases as furnished by my assistant G R Visvanathan who was deputed to collect materials is as follows —

Subject —Bullock O P 785 of Tanjore hospital
Colour —White Age 4 years
Breed —Country bred (Pulikulam breed)

History of the case —This case was admitted on the 28th October 1927 as an out patient During the time of admission it was reported that the animal had noisy breathing and there was some obstruction in the nostrils When examined by the Veterinary Assistant in charge there was a piece of growth seen in the left nostril. The growth was situated in the anterior portion of the nasal chamber It was pedunculated and protruding out during expiration and retracting during inspiration Even during normal respiration the breathing sound was heard at a distance The site of the growth was a little behind the nose string hole and was attached to the Schinedarian mucous membrane to its outer aspect Its pair has not developed the disease

Subject —Cow O P 834 of Tanjore hospital
Colour —White Age 6 years
Breed —Country bred.

History of the case —The owner states that he first saw the growth in the nostrils while the animal was drinking water

Symptoms observed by the Assistant —

There was a small pedunculated soft cauliflower like growth in the right nostril of the animal The growth was limited to the anterior portion of the nasal chamber and about the size of a bean and attached by means of a slender fibrous like material to the Schinedarian mucous membrane on its outer side While the animal was breathing the growth was pushed forward and was visible even from a distance and during inspiration the growth was drawn back There was mucous discharge from the affected nostril. The breathing was slightly noisy and heard at a short distance When manipulated with the finger the growth was found limited only to the anterior part

Explanation of Plate XXXIII

Fig 1 Growth showing its papillose character with fine bands of tissue On its surface are seen minute spots which are the sp

„ 2. Epithelium is seen thrown into folds Numerous sporangia under the surface epithelium.

„ 3 (*A*) The surface epithelium is seen becoming very thin and at are seen fully developed sporangia about to rupture

(*B*) Rupture of the epithelium and of a sporangium with the spores are seen

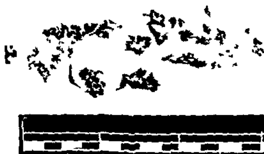


Fig 1



Fig 2

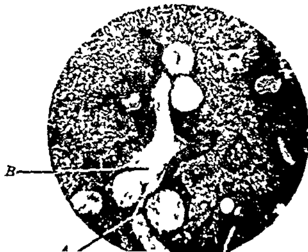




Fig 4



Fig 5

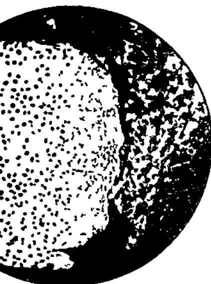


Fig 6



Fig 7

Explanation of Plate XXXIV.

Figs 4 and 5 Sections showing the cell wall of the sporangia varying in thickness and surrounded by leucocytes

Fig 6 Section shows sporangium in which fully formed spores are seen in the centre and small ones at the periphery

" 7 Section showing two Sporangia in which fully formed spores are seen at one pole

DISCUSSION.

Mr J T Edwards (United Provinces) The observation of infection with rhinosporidiosis in the lesions of the so called infectious nasal granuloma of cattle in India has also been demonstrated to me at the Congress by Col Knowles, I M S I am familiar with Krishnamurti's writings upon this disease condition and have also some experience in its investigation at Muktesar The appearance of the curious granules with a structure simulating that of the 'ray fungus' in actinomycosis and the histological characters of the lesion tended to the conclusion that the disease was similar in origin to the bovine actinomycosis of Western countries (caused by Nocardia or Actinobacillus) In his writings, I understood Mr Krishnamurti to state that the disease was streptothrical in origin The causal streptothrix has not, however, been cultivated yet with certainty Although at Muktesar we have very readily isolated such organisms from lesions, we have not been able to transmit the disease by means of the cultures, or, in fact, in any other way artificially

It now seems to be the opinion of Mr Krishnamurti that the disease condition is set up also by Rhinosporidium, also now generally regarded as a fungus, so that further investigation to clear up the question of causation is required

It is curious that, from the hitherto unpublished records of Rai Sahib P N Das, Civil Veterinary Department, Bihar and Orissa, the nasal granuloma remains unaffected after treatment of animals with iodides, in the manner commonly adopted in actinomycosis but yields readily to repeated intravenous injection with tartar emetic

Mr V Krishnamurti Ayyar (Madras) replied Mr Edwards says that I have omitted to mention the work done by others on nasal granuloma, especially that of his colleague at Muktesar I would like to mention that my paper is on 'Rhinosporidiosis in Cattle' and not on nasal granuloma due to a streptothrix I only pointed out in my paper the macroscopic and microscopic differences of the growths due to a Rhinosporidium and to a streptothrix which was first described by me in 1922 as being the causal agent of nasal granuloma in cattle

GASTRO-ENTERITIS HÆMORRHAGICA IN THE CATTLE OF FORMOSAN MILKERS

BY

TOSINOBU MIYAMOTO,
TOSITUNA NOMURA

AND

SIWITI ONO,
Government Research Institute, Formosa, Japan

Outbreak

Since the last ten years an acute gastro enteritis among cows, chiefly of the Holstein hybrid breed, has been noticed leading to the loss of about 200 head

Course and Prognosis

One hundred and eighty seven cases observed, severe cases take a per acute course and death occurs within 24 hours, the acute cases die within a week, light cases may recover in five to ten days Mortality is about 43 per cent

Symptoms

Thirty five cases observed, high, quick and irregular pulse are marked terminating with acute course without exception, fever not marked, respiration quiet Neither vomiting nor colic, appetite impaired, constipation, diarrhœa watery, mucus or mixed with blood.

Anatomical Changes

Twenty cadavers examined Chiefly digestive canal and heart are affected. Jejunum, ileum and cæcum are most noticeable, next the colon and stomach. Hæmorrhage of mucous membrane, necrosis, erosion or separation of the epithelial layer and also hæmorrhagic infiltration of the submucosa Heart with extensive hæmorrhages, especially the auricles, apex, valves and other parts of the epi- and endocardium, but not extending very deeply into the myofibre

Micposcopical

Digestive apparatus, blood congestion hæmorrhage, round cell infiltration into the mucous membrane, submucosa and muscle layer degeneration necrosis and erosion of the epithelial layer Heart, blood accumulation in the loose connective tissue or superficially but sometimes intrafibrial, hæmorrhage of the heart muscle recognized by cloudiness and degeneration of the myofibre

Causal Organism

By intravenous injection and feeding experiments with pure culture of *B paratyphosus*—B type like bacillus isolated from the bloody mucous fæces of two cases healthy test cattle become sick with symptoms and anatomical changes identical with those seen in natural infection If the introduced bacilli are small in quantity the experimental infection is light and the animal recovers with a larger quantity the animal develops a severe infection with marked symptoms and a fatal termination

Modf of Infection

Consideration suggested the food material called 'Tofukasu' which consists of ground raw bean When this material was fully disinfected by hot steam, the outbreak was brought to a standstill

Conclusions

We have discussed systematically the hæmorrhagical gastro enteritis of cattle outbreaks of which have occurred during the last ten years among the cattle of milkers causing much loss We have isolated a special infectious agent which belongs to the *B paratyphosus* group and resembles its B type but which is not the same Finally we have brought the disease to a standstill by a simple practicable means

UROCYSTITIS HÆMORRHAGICA OF NATIVE CATTLE IN FORMOSA.

BY

MAJOR TOSINOBU MIYAMOTO

Veterinary Surgeon, Government Research Institute of Formosa

FROM olden times the reddish urine of the Zebu breed of cattle has been noticed by Formosan farmers and several primitive therapeutics have been devised although some investigators do not attribute this feature to disease

The prefecture of Shinchiku annually records about one hundred cases but the other districts are less affected For several years research work has been undertaken on cattle discharging red urine both in the said prefecture of Shinchiku and other districts of Formosa

The feature has been investigated clinically pathologically and ætiologically and from my personal researches supported by infecting experiments I recognize that this red urine does not coincide with the Stallroth it is also definitely distinct from tick fever or other hæmoglobinurias caused by protozoal infections neither is it caused by such parasites as bilharzia filaria etc The disease usually takes a chronic course the body temperature normal general condition and appetite not appreciably affected except after the second infection the animal even submitting to light labour It is generally concluded that the red urine almost always arises from cystitis with or without tumefaction At the fourth congress of the F E A T M at Weltevreden in 1921 I presented the result of my research under the caption of Hæmaturia in the Cattle of Formosa and Dr de Moulin stated Prof Wester explains hæmaturia of calves as a reflex action of the wall of the blood vessels after drinking cold water the general pressure in the blood vessels increases and the consequence is that the smallest vessels in the kidneys burst'

Thereafter I continued my researches in this subject until 1923 thus giving five years attention to this matter Believing that all possible varieties have been practically under observation I intend to briefly tabulate the resulting pathological changes of the cystitis hæmorrhagica

CLASSIFICATION OF UROCYSTITIS HÆMORRHAGICA HÆMATURIA

Based on 141 cases clinically observed of which 74 cadavers were dissected and of these 74 60 were microscopically investigated moreover several cyst

ecimens were received from the slaughter house, which indicated traces pathological changes, although there had been no apprehensible symptom fore slaughter The so called 'hæmaturia' should be naturally classified as lows —

1 —*Simple and genuine hæmaturia*, believed by the owners to be healthy licating no symptom of sickness to the unaided eye, appetite good urine of rmal colour, this class includes —

(a) Slight acute cystitis with some infiltration and inclination to hæmorrhage l growth of cyst wall Blood corpuscles included in the urine proved micro pically only with trace of protein reaction Many such cases were disregarded their owners

(b) Cystitis acuta recidiva Such acute cystitis as above having repeated apse, resulting in permanent growth in the cyst wall indicating that inflammation vailed

2 —*Light blood tinged urine* which is acknowledged as abnormal but not iously considered by the owners who generally neglect this feature which ers sub groups

(c) Cystitis acuta of fairly high degree Urine becoming red and dim, blood ication visible to the naked eye but after some duration this passes off and the kness naturally passes off

(d) Cystitis chronica recidiva with inclination to progressive growths This nent chronically repeating indicates the presence of light invisible hæmaturia in sub group (a) or (b)

3 —*Hæmaturia cystitis* to which owners pay increased attention

(e) Urine deep red and dim the colour sometimes changing (deep or light) ording to the degree of labour imposed or to the condition of temperature or son of the year Though it may sometimes be remittent or even intermittent levelops into recognized stable hæmaturia i e chronic neoplastic cystitis with ady primitive growth of benignant or malignant tumour or even concrete nours

(f) Primary and also secondary complex or simple growths develop in the t, accompanying the degenerative changes and necrosis of some parts of these wths also at times developing croupous or diphtheritic pseudo membrane inside cyst The urine becomes extremely dim and of dirty brown colour, with much iment consisting of necrotic detritus, pseudo membranous fragments, blood gula and some salts etc

4 —The course of *hæmaturia* is liable to sudden change with accidental nplications Several diseases complicate hæmaturia for example bovine uenza, pneumonia gastro enteritis and the increase of endoparasites, especially r migration and also chronic cases are changed to acute by dents such as the s m a height, y tumble

5.—*Hæmaturia of cows provoked by pregnancy* Pregnancy occasionally causes hæmaturia in a cow especially in one which has experienced repeated parturition Hæmaturial cows dropping healthy calves year after year develop the disease which increases proportionately with the successive parturition, although the calves appear perfectly healthy

6.—*Hæmaturia of buffaloes*

Generally met with only in the males and oxen very rarely, or practically never in female buffaloes The cause is almost invariably retentio urinæ, by which the cyst enlarges, congestion and stasis in the circulatory apparatus of the cyst wall extravasation and hæmorrhage in mucosa and submucosa take place The retained urine increases its volume by the admixture of the blood quantity finally resulting in ruptura cystis and death The cause of retentio urinæ is in all cases, the lithiasis pyelis

The calculi formed at the pelvis renalis which are generally grain shaped, though not so large (but usually numerous) descend gradually to the bladder, enter the urethra, and reach near the penis orifice where the canal is comparatively very narrow and does not yield to distention The calculi are there arrested as emboli blocking the passage partly or wholly

Some Statistics relating to the Occurrence

I.—*Division by Ages*

Mentioned 37 examples containing 5 bulls 32 oxen

Ages	Number of patients	Ages	Number of patients
			B F 13
1	1	7½	1
4	1	8	4
4½	1	9	3
5	3	9½	1
6	1	10	6
6½	1	11	4
7	2	11½	1
I	2		3
	1		1
	13		

B —*Cystitis with tumefaction Twenty one examples mentioned*—concld

Changes	Animals	No	Ages
Brought forward		13	
(8) Carcinom and papillom	Ox	1	8
(9) Young carcinom	Cow	1	7
(10) Spindle, polymorph cell sarcom and papillom	Ox	1	10
(11) Young sarcom and carcinom	Ox	1	11
(12) Polymorph cell sarcom and papillom	Ox	1	0
(13) Superficially carcinom deeper part sarcom	Ox	1	10
(14) Small cell sarcom	Ox	1	10
(15) Polymorph cell sarcom	Ox	1	13
TOTAL		21	

III —*Relation between pregnancy and hæmaturia*

A Hæmaturia in pregnant cow — 4 cases

(a) 8 years old — Carcinom in cyst
(b) 9½ years old — Papillom
(c) 4½ years old — Cystitis hæmorrhagica
(d) 7 years old — Productive cystitis

B Hæmaturia with parturition or without it

(a) 7 year old cow, hæmaturia from 2 months after parturition
(b) 1½ year virgin cow temporary hæmaturia
(c) 1½ year Indian hybrid temporary
(d) 8 cases passed one or more parturitions
Their ages 4, 4½ 8 11, 9, 9 10 10

IV —*Blood relationship*, one example

Six cattle belonging to the same owner bred and reared in the same stall

(1) Mother cow
(2) First female calf 1 year and 7 months old
(3) Second female calf, one year old
(4) First grandson that is, the calf of (3)
(5) Sire of (4)
(6) Second grandson, that is, the younger brother of (4)
All the above were attacked excepting the last one (6)

V —*In same stall* two cases

(1) Farmer R's native ox 10 years old died on 10th June 1919 papi carcinom ulcus and rupture are seen in cyst The owner introduced an young bull 5 years old to fill up the vacancy in the stall but after 1 year the one was slaughtered on account of hæmaturia *post-mortem disclosed pap* in cyst but this may have originated prior to the introduction of the ox the stall

(2) Farmer Y s ox bred in his own stall on 9th September, 1915

From September 1919 he discharged red urine without any noticeable c Another ox of the same owner bred in 1913 also began to discharge bloody u from March 1921 and so on

The Classification of the Pathological Changes of Urocystitis Hæmorrhagica

Sixty examples collected under considerable difficulty by myself are reco with minute protocols of which 40 cases were observed at post mortem especially the bladder and other organs related to it are discussed microscopic by preparing the numerous double stained preparations for each example full demonstration of pathological changes for each example was most impor and difficult but it is impossible to cite them all here for this paper will permit nor would I be able to hold your interest Excepting two cases which hæmoglobinæmia and another two which had hæmorrhage caused by retentio u and ruptura cystis in a carabao the remaining 36 examples might be classifie two principal groups

I Urocystitis without tumour covers 12 examples

II Urocystitis with tumour covers 24 examples

Of course there is no exact demarcation between these groups indicat gradual transition also there are several degrees of change amongst the gro themselves for example incomplete tumefaction only simple preliminary cor tion of tumour progressive hypertrophical state of epithelial cells and so on

Moreover there are not only simple or complex forms of tumours but t they are increasingly complicated by secondary invasions or partial regress degenerations

Subdivision of 1st Group

I —*According to the locality of pathological change*

(*a*) Whole surface of mm of cyst general change	8 cases	circa 67 per ce
(*b*) Chiefly the fundus of cyst is changed	2 cases	circa 16
(*c*) Remarkable changes from fundus to apex	1 case	circa 8
(*d*) Remarkable change in apex	1 case	circa 8

II — *According to the size of cyst or quantity of content*

(a) Somewhat shrunken, emptied state	2 cases	16 per cent.
(b) Dilated	4 cases	33 „
(c) Somewhat enlarged	1 case	8 „
(d) Fairly enlarged	4 cases	33 „
(e) Widely enlarged	1 case,	1 „

III — *By the degree of pathological changes*

(a) Cystitis simplex	2 cases	16 per cent
(b) Cystitis hæmorrhagica	4 cases	33 „
(c) Cystitis hæmorrhagica purulenta et pseudo membranacea	1 case	8
(d) Cystitis hypertrophica et progressiva	4 cases	33 „
(e) Cystitis diphtheriteca, pseudo membranacea cum psammos	1 case	8 „

Subdivision of 2nd Group

I — *By the locality at which the growth exists*

(a) The fundus and triangular part of cyst Papillom, 3, Sarcom 4, Carcinom 7	14 cases	58 per cent
(b) Generally diffused in whole cyst Papillom, 1, Sarcom 1, Carcinom 3	5 cases	21 „
(c) From fundus to apex . Papillom 2 Sarcom, 1, Carcinom, 1 the carcinom occupying about four fifths of the surface of mm	4 cases	16 „
(d) Scattered irregularly Papillom, 1	1 case,	0 8 per cent

II *By the degree from simple and benignant to complex malignant*

A *Papillomatous growth complete papillom carcinomatous growth*

(1) Papillomatous hypertroph, 1 case

(2) Bush like numerous papillom growths and connective tissue hypertrophy, papilliform, callositas like, and nodulous new formation thickness, 2 5 cm, 1 case

(3) Papillomata one large and several small hanging with stem or cord, 1 case

(4) Papillomata, two large and 16 small hanging 1 case

(5) Gland cell papillom thickness, 2 or 3 cm, 1 case

(6) Papillom and carcinomatous growth containing lymphomatous part, hanging with stem or making protuberance thickness, 2 5 cm, 1 case

(7) Papillom and papillomatous growth of prostata, partly carcinomatous growth and angiom with stem and protuberances, thickness, 3 cm, 1 case

(8) Young carcinom and papillomatous growth, thickness, 0 5 to 2 0 cm 1 case

B *From progressive hypertrophic cystitis to carcinomatous growth, and fro papillomatous hypertrophy to the carcinom*

(9) Young carcinomatous hypertrophy, thickness, 1 5 cm , 1 case

(10) Young carcinomatous growth thickness, 2 0 cm , 1 case

(11) Carcinomatous growth, partly ca durum, large ulcus formation, thicknes 1 3 cm 1 case

(12) Carcinom and papillomatous hypertrophy, thickness 1 0 to 4 0 cn 1 case

(13) Carcinom, flat progressive infiltrated form, thickness, 1 0 to 4 0 cm 1 case

(14) Carcinoma durum thickness 1 5 cm 1 case

(15) Carcinom, thickness, 2 0 to 2 5 cm 1 case

(16) Carcinoma durum, thickness 2 5 cm , 1 case

(17) Carcinom and large ulcus formation, thickness 3 0 cm , 1 case

(18) Carcinoma durum, papillomatous growth, wide and hard connecti tissue cell hypertrophy including fibroma and sarcomatous parts, thicknes 3 0 to 5 0 cm , 1 case

C Progressing hypertrophic cystitis develops to benignant and more ofte to malignant tumour traceable by the malignification of the tumours an developing to the complicate growths of two or more malignant ones

(19) Both young carcinomatous and sarcomatous growths, thick hypertroph form, 1 case

(20) Small spindle cell and polymorph cell sarcom, and papillom which consi of baby head large protuberance and several cm long polypoid or fimbriate membrane 1 case

(21) Superficially carcinom, deeper part large spindle cell sarcom, and ulcu that is a papilliform, protuberant and ulceration form 1 case

(22) Superficially round cell or polymorph cellular sarcom, deeper part chiefl spindle cell sarcom, the whole making a large cauliflower (blumenkohl) lik protuberance 1 case

(23) Superficially plastic granulating cells, the deeper part long spindle cel sarcom partly making a large papillom covered with dense fimbrial or polypou growths and imbedded myxom and an angiom

The whole are large complicated malignant growths

(24) Small round cellular sarcom making gigantic growth and metastating and connecting with surrounding organs, 1 case

ÆTIOLOGY OF CYSTITIS HÆMORRHAGICA OR HÆMATURIA

Grouping and classifying all the recorded cases, and speculating as to their pathological changes, I can prove with confidence, that each case is correlated with

the others as to their causal relations Therefore, based upon the origin of cystitis and tracing the degrees of the pathological changes I make the following proposals —

(1) The causal agents or data of two or more malignant growths (e g the complication of carcinom and sarcom) may be at the same time also those of the two or more complicated growths of malignant and benign growths

(2) The causal agents or data of malignant and benign complex growths may also be those of the simple malignant growths

(3) The causal agents or data of the simple malignant growths may also be those of two or more benign complicated growths

(4) The causal agent or data of the benign complicated growths may also be those of the simple benign growths

(5) The causal agent or data of simple benign growths may also be those of chronic hyperplastic cystitis

(6) The causal agent of chronic hyperplastic cystitis may also be that of acute simple cystitis

(7) The causal agent of simple cystitis may also be that of slight temporary cystitis which cannot generally be discriminated by the owners

Reversely the causal agent or data of slight not easily discernible temporary hæmaturia can cause several other and graver sicknesses and remarkable changes by acting repeatedly or continuously

Throughout the above proposals 1 to 7 or from stadium prodromorum of tumefaction to throughout the succeeding groups to the primitive true tumours the small or large benign or malignant, simple or complex forms and so on the causal agent or data may act commonly, and run through the various numerous cases Regarding the origin or cause of malignant tumours the celebrated Virchow s 'Irritation Theory has been proved, in 1917, experimentally by Drs Yamakiwa and Ichikawa by means of the artificial carcinom produced on rabbit s ears with tar by which they established the history of carcinom evolution in concrete form, which need not be repeated here Dr Yamakiwa's students also succeeded in producing artificial sarcoma by irritation

My researches naturally do not serve to show the history of the formation of tumours in one and the same animal as my experiments were confined to the inner organ entailing the sacrifice of one animal for every specimen But my investigations and experiments extend over four or more years (1918 to 1921 and 1922) in the same restricted section of Formosa, relating to 141 patients, and numerous pathological exhibits of bovine cysts I can arrange and compare these specimens according to the gravity of their pathological changes in the cyst wall, macroscopically and microscopically, i e from almost normal and only slight hyperplasia of the cyst wall to several exhibits of inflammation with or without tumefaction These specimens arranged parallel to the recorded history of the

rabbit test referred to afford ample corroboration excepting that my examples are from an inner organ in which the cyst can only be disclosed on the sacrifice of the animal while in the rabbits the tests being on the outer organ of the ear can b observed daily and recorded accordingly Moreover the causal agents in my bovine investigations were natural ailments or data while in the rabbit tests the unnatural irritant of tar was employed Moreover in the cases investigated by me the agent or data which might of themselves be the cause of simple ligh *cystitis hæmorrhagica will in time develop into or give rise to true tumours* through their repeated or continuous irritation Also the same trivial agent or data may change the benign tumour into a malignant one and more complex and malignant developments may result from secondary agents

The demarcation between the above classified groups and also those between the sub-classes of the same groups are not sharp or clearly defined evolving very gradually from light to grave from simple to complex and so on But the rate of *gradual change is not equally applicable to each cystitic stage on account of the* pathological changes influenced in general by the pathogenic agents or condition circumstances or physical condition of the animals Several variations arise inequalities such as acute and grave hæmorrhage early suppuration chronic new formations a solitary large papillom numerous small papillomata early ulceration inclining to carcinom formation the chief lesion limited to epithelial layer early development of sarcom in the submucosa superficial and profound hæmorrhages and progressive growths in cyst at the same time etc

On the other hand the property of the urine in patients is itself unstable the ingredients varying in quality also the secondary irritating agents either micro organisms or chemical substances are not at all times the same Even in one and the same *animal the irritating materials vary by season or by circumstance* so that during a long chronic case several different irritants can appear at the same time or consecutively The irritants are not always necessarily of a severe character even mild irritants may give rise to the several kinds of pathological changes including very malignant and complex growths in the cyst by long continuous action

I can adduce with conviction that the causa irritans of hæmaturia is or is not of a certain kind need not be limited to only one may be double or more there may be one or more secondary contributory causes

(1) Micro organisms suspended in the cyst urine meeting a good nutritive medium in it decompose the medium producing certain materials which irritate the mucous membrane

(2) The animal accidentally takes up some irritable matter or toxic herb with its food

(3) Certain harmful decomposing or irritating products of the animal s own system which are discharged with the urine during a state of fever may not *only* cause inflammation in a secondary manner but may at the same time cause retentio

urinæ by the paralysis of m accelerator urinæ or by the spasm of m sphincter vesicæ

In addition endoparasites or mechanical trauma from outside come into consideration and I can enumerate the following facts which may possibly have some significance ætiologically —

(1) *Micro organisms* —Immensurable bacteria especially diplo streptococci in the patient s urine

(2) *Feverish disease* —An influenza like feverish disease of cattle prevails in several villages in Shinchiku prefecture by rotation It is not fatal recovery occurs within a week or ten days more or less The ætiological research of this disease is not yet completed

(3) *Ophthalmitis* —In the hæmaturial affected locality there occasionally prevails a kind of cattle ophthalmitis It is not yet proved that there is any ætiological relation between this eye affection and hæmaturia

(4) *Scanty grass* —The hæmaturial localities are mainly mountainous intersected by valleys and gravel beds while the tillable lands are often narrow limited terraces or slopes poor in grass but also scanty in toxic herbs

The grazing cattle are fatigued and in bad condition throughout the seasons condensed urine is frequently retained for a long period as opportunities for urination are checked

(5) *Trauma* —Caused by blows or the straining of the cyst or other abdominal or pelvic organs through rolling over banks wandering on very uneven ground clambering down irregular steep tracks and stumbling about rocky grazing ground

(6) *Weather* —Windy locality Temperature not so high as in other localities Soil largely consists of delugeal gravel dense hard mud and red acetic soil

Water soft and clear the cattle drink from springs brooks and ponds

(7) *Parasites* —Seen in sections or microscopic preparations these may not seem to have much ætiological significance but the following have been noted —

Larvæ of *Filaria labiopapillosa* in immensurable dimension multiply among the hydroperitoneum causing peritonitis and a number of them penetrate the cyst wall the lymph glands in the abdominal cavity the cyst changing entirely into a large sarcom

Larvæ of *Filaria labiopapillosa* imbedded in the carcinom tissue of cyst Also *Fasciola hepatica Eurytrema pancreaticum Filaria lacrymalis Homologaster taiwana Amphistoma conicum* and in one example a renopelvic calculus Special features were *Schistosomum japonicum* and its eggs in the venaporta venamesenterica venarecti but not in the cyst nor in the urine *Nephrophagus urine* has been seen in the urine After careful consideration it was decided that these parasites are not essential causal agents and may be merely accidental concomitants

In short one special causal agent, organismal or material, has yet to be determined Even those instances in which the immediate causes have been ascertained there is no definite indication upon which to emphasize a satisfactory conclusive causal agent

On the other hand, slight cystitis is not dependent upon a special infection, nor upon an irritating agent nor yet upon a violent accident, as its cause Abundant diplococci and other bacteria are always detected in the hæmaturial urine or found in the preparations and even in the tissues may be the cause of the inflammation or multiplying as the result of inflammation, may co operate mutually

On the contrary even in healthy cattle bacteria will be found even in normal urine as is proved by numerous control cases But normal bovine urine is not a good nutritive medium for bacteria, it rather checks the multiplication of bacteria Nor does the healthy mucosa of cysts readily admit of the invasion of bacteria Certain circumstances or conditions, however weaken the resistance of the mucous membrane of the cyst and may cause lesions in it The inflammation of the cyst may even be caused by common substances or agents which are present in the urine and easily changeable

Finally I may summarize the above conditions or circumstances as follows —

Bovine influenza, seasonal and local

Geographical situation, mountainous intersected with valleys and gravel beds

Weather conditions, windy

Scanty grass poor grazing

General living conditions of the cattle half stall and half grazing or wholly grazing

Infection Experiments

(1) *Patient animals as antigen* —Five diseased Formosan cows were introduced by one from their native locality to Taihoku one of them died in Taihoku station on arrival, the other four survived for two weeks up to six months or more and supplied their bloody urine as required

(2) *Healthy animals for infection* —Holstein hybrid calves male and female, rabbits guinea pigs, and a native bull were used these animals were injected to their emptied cysts with fresh bloody urine or diplococcus emulsions which were isolated and cultured from the urine and sometimes with both together

(3) *Kinds of practice* —(*a*) Observation of the animal as to general condition *especially the colour quantity, frequency and resistance of the urination*

(*b*) Search for bacteria and spirochæta from the hæmaturia The centrifugal sediments of the patient s urine examined through dark field apparatus or the smears of the sediment with Giemsa staining or silver gilding method

(*c*) Agglutination and complement absorption of two kinds of diplococci

(*d*) Artificial urination of healthy animals and bloody urine injection into the emptied cysts

(*e*) Auxiliary means to urge the invasion into healthy animals

(*f*) The existence and distribution of micro organisms in the tissue of cysts

(4) *Artificial urination from cow*—Experimenting with several kinds of atheters and the uterine speculum or rectospeculum usually employed for horses cows dogs or women after great perseverance and repeated failures I at last succeeded in making a newly designed catheter which is a metallic pipe plated with nickel about 15 inches long its diameter equal to that of the horse Toward the end it is slightly crooked and on both sides near the end there is an oblong hole the so called eye at unequal distances from the end The peculiarity of the instrument is the slight curvature of the whole which was decided upon after repeated experiment and the fact that it is not made of rubber

Its method of use is as follows - Open the vagina with a woman s uterine speculum inspect the orifice of urethra and insert the catheter This work is not so easy at first but after repeated training there is no necessity to use the vaginal or uterine speculum urination will result from tickling the part with the catheter end After much discipline the cow understands what is required of her and soon readily discharges her reserved urine under the preliminary motion of catheter management

At last by approaching and calling the cow while wearing the work uniform and carrying the urine vessel and instrument she will even just after a generous urination bow the loin to discharge a few drops of red water from the almost empty cyst

(5) *Cyst injection to the healthy calf*—Open the vagina with a rectal speculum or by the finger of the operator or assistant inspect the orifice of urethra and insert slowly the newly designed five metallic catheter

There is usually some resistance through the contraction of the urethral muscle Stop the insertion when the orifice of the urethra is reached and connect the catheter with syringe Never insert more deeply into the cyst being careful not to penetrate the cyst wall by the catheter

(6) *Artificial urination of the diseased bull or ox*—Red urine is drawn not only from a sick cow but from a sick bull or ox For the bull the catheter is not to be applied so the following methods rise for consideration —

A The contraction of cyst by abdominal pressure caused by the animal himself In order to get the animal which is commonly trained to pull a plough or waggon to discharge at such a time as we desire, fit the utensil to the animal s body and urge the animal to go on with heavy ploughing by oral encouragement repeatedly, then the animal becomes in shape and position to draw with both hind

legs extended backwards and thereby tightens his belly, soon after he begins to make water It is also possible to get good results by pouring water suddenly on the rump of the animal, for the same purpose

B Irritation of urethra It is most important at first to know fully the natural evacuation or discharging process of each animal I have watched the patients continuously through many hours with a vessel ready to receive the discharges Meanwhile I succeeded in getting urine by such irritation of urethra or penis as rubbing along the urethra course or the penis sheath from above towards the end but by this method the spermatozoa are often mixed in the urine

(7) *To drain rabbits and inject into them* —In rabbits male or female equally the rubber catheter is inserted and the urine flows out through it and the same inserted catheter may be connected with the syringe for injection purposes

(8) *Cyst injection for bull or ox* —It is not possible to reach entirely up to the bladder by injecting the antigen material from the end orifice of the urethra by inserting a fine rubber catheter or the long beak of a syringe because the course is long inflected three times acutely, and is in the ascending direction against the push of the animal Therefore urethrotomy is employed in order to complete the injection at the part of ischiatic bending of the urethra

Experimental Results

(Details of the experimental processes have been omitted)

(1) *Micro-organisms in the tissue of the cystitis with tumour*

Examined animals were 11 cattle and 1 rabbit which were infected naturally or artificially Bacteria present

(a) Chiefly in the mucous membrane	1 example
(b) Much in the part of hæmorrhagic inflammation few in old indurated part	1 "
(c) Diplo and streptococcus contained intra epithelial cellulare of cyst tumour	1

Note —The detected bacteria were diplo and streptococci which in a few examples were accompanied with a bacillus

(2) *Micro organisms in the bloody urine*

Native cattle No 1 —Female 8½ years, with productive cystitis and hæmaturia Observed duration 6½ months from 23rd November 1921, to 6th June 1922 examined 67 times always detected

Hæmolytic diplococcus 1 kind

Native cattle No 2 —Female 8½ years with productive cystitis and pseudo membrane formation hæmorrhagic purulent nodulous nephritis, and

hæmaturia Duration 81 days Examined 30 times Detected non hæmolytic diplococcus 1 kind Diplo streptococcus 1 kind

Native cattle No 3 —Female, 8 years with hæmorrhagic inflammation of submucosa and papilloma Duration 12 days Examined 8 times Detected non hæmolytic diplo and streptococcus

Native cattle No 4 —Male 2½ years with carc noma durum Duration 75 days Examination 10 times Detected hæmolytic diplococcus

Contrast example The urine of healthy cattle 3 water buffalo 1 and Holstein hybrid 1, were all negative to the above diplococcus Spirochæta were never found in the urine of all above animals

(3) *Agglutination*

A *The agglutinating rate* of the serum of diseased cattle to the diplococcus

Serum of	Dilution times	Results
Native patient	320—640	Positive
Native healthy	160—320	Negative
Holstein hybrid	80—160	

It seems that the diplococcus has little significance as one of the causal agents

B *Complement absorption* —The antibodies do not absorb the complement, the ætiological meaning of the diplococci is therefore negative by this method

(4) Infection by the injection of bloody urine or the isolated diplococcus into the healthy bladder assisted by the cyst injection of $AgNO_3$ or tinctura cantharides and by the cooling of the part

A *Injected materials* —Five kinds of bloody urine originating from five patients three strains of diplococcus in suspension emulsion or in nutritive media

One kind of healthy urine which contains a few mono or diplococci

B *Healthy animals for injection*

Native cow 1 year old	1
Holstein hybrid calves	3
Rabbits	9
Guinea pig	3
TOTAL ..	16

Details of the protocol post mortem results and microscopic changes are omitted here the results being summed up as follows —

Injected animal	Injections	Duration of examination	Termination of animal	Result.
Rabbit No 1	8	40 days	Survived	Positive
" " 2	91	Circa one year (Dec 30,'21—Oct 13 '22)	Death	Negative
" " 3	21	3 months	"	"
" " 4	25	94 days	"	Positive
" " 5	21	6½ months	Killed	Negative
" " 6	6	95 days	Death	"
" " 7	17	44 "	Survived	"
" " 8	21	47 "	"	"
" " 9	2	14 "	"	"
Calf " 1	2	55 "	Death	"
" " 2	21	25 "	"	Positive
" " 3	15	45 "	"	"
Guinea pig " 1	1	22 "	"	Negative
" " 2	9	40 "	"	"
" " 3	8	30 "	Survived	"

Summary of the above results

Rabbit No 1 By section, bladder enlarged, and hæmorrhagic inflammation of the mucous membrane

" " 2 Obstinately resisted artificial infection, clinically slight cystitis, not dissected

" " 3 Death by coccidiosis, examination discontinued

" " 4 By section, congestion, of the m m of bladder and diplococci contained in the epithelial cells

" " 5 Clinically negative, examination stopped, not dissected

" " 6 Death by septicæmia, examination discontinued.

" " 7 Depressed by the injection but recovered soon after ceasing the injection

" " 8 Clinically negative, examination stopped, not dissected

" " 9 Body weight diminished gradually but negative in post mortem

Calf " 1 Not dissected, the cause of death not related to the injection.

Plate XXXV

Fig 1 Hæmaturia native ox 18 years old Exampl No 80 Photo three days before death
Red urine continued 1st November 192 to 27th February 1923
Post mortem papilloma and productive inflammation of cyst

Body height wither	1 45 m
sacral	1 34 m
Body length—(horn root—ischia end)	[illegible]18 m
„ (shoulder—ischia end)	1 58 m
Girth	[illegible]01 m
Loin circumference	1 8 m
Depth of chest	0·86 m
Length of face	0·561 m
Aperture of eyes	0·[illegible]31 m
horns	0 13[illegible] m
Thickness of left meta corpus	[illegible] m

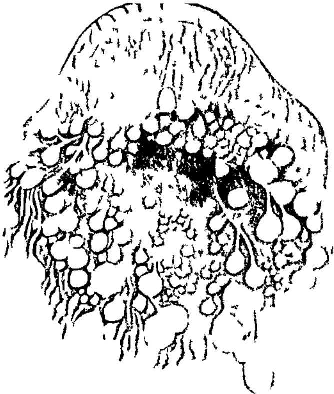


Fig. 2 Hæmaturia example No 9 *Cystitis ulcerosa*
Cyst opened and mucous surface exposed The mucous surface very rough with many nodules and ulcers of different size and hæmorrhagic infiltrations
A deep and large ulcer 3 × 1 5 cm with irregular border



Fig 4 Hæmaturia Carc nomatous growth Ins (vew of y





I g 6. Hæmatu a example No 7 Cy t n
Se tion su face aul fl wer l ke growtl 7 11 10 up al y wh te
w th hæmorrha c spots The muscle lay s of yst are p t y l ma ate
fr m s l pa tly in n xed w th the no



F f n a a l N 18 l a p o natous a l sarco natous
w w th ate l polyp l an ng ov r a l the mucous
t The n ucous s de of cyst expos l

Calf No 2 By section congestion and round cell infiltration of the mucous membrane and the inclination of productive hypertrophy of epithelial cells and submucous connective tissue

" " 3 By section excoriation of cyst epithelium, partial growth of mucous membrane round cell infiltration congestion and extravasation of blood in the tissue

Guinea pig No 1 By section bladder enlarged also kidney hypertrophic, but no pathological changes of the bladder as yet

" " 2 Death by a cause not related to the injection

" " 3 Examination discontinued, negative

SUMMARY

(1) It is very difficult to produce cystitis in healthy animals by the cystal injection of bloody urine itself which contains abundant diplococci or other micro organisms, or by their pure culture infecting the mucous membrane of cyst with the micro-organisms and other ingredients of the bloody urine The normal mucous membrane of the cyst has a very strong self protective resistance against the invasion or may soon recover from the slight lesion and the normal urine has usually some controlling power against certain injurious micro organisms

(2) Two kinds of diplococcus isolated from the patient's urine temporarily named 'Diplococcus hæmaturiæ bovis' have no special ætiological meaning compared with other micro organisms and other urine ingredients But because these two kinds are most abundant and common and they are also found occasionally in the tissue or intracellularly in the inflammatory m m and the growth of the cyst it may be said that they may be more serious agents than several other changeable and unstable micro organisms and ingredients and may at the time promote the cystitis

(3) There are several factors (*see* above) which may contribute to cause the cystitis, but the final and actual irritant of the urocystitis hæmorrhagica has not yet been decided

CONCLUSIONS

This research work covers a wide field in which the writer has exerted his utmost effort in the pathological anatomical researches

(1) By observing from several standpoints he ascertained that the hæmaturia which is seen in the native yellow cattle and water buffalo is different from the hæmoglobinuria which has been seen hitherto in the cattle, and also from the hæmaturia which develops from some other cause

(2) Based on the large number of hæmaturia patients, and especially on the anatomical changes seen in 56 examples he makes clear the disease by establishing that the hæmaturia originates from hæmorrhage of the mucous membrane of the cyst where the pathological changes take place primarily

(3) From the substantial sickness of the cyst it is to be acknowledged that there exist several morphological changes such as cystitis, and, moreover, benign and malignant new formations (continuing from undeveloped to developed cases) Moreover, it is confirmed by the writer's careful histological inspection, (*a*) that the starting point of these various changes is to be attributed, after all, to inflammation of the mucous membrane of the cyst, *notwithstanding that the* changes seem to be so very rich in variety, (*b*) that when once the cystitis occurs by whatever cause the hæmaturia may appear and, if the cause is chronic, then the new formation goes on, (*c*) that the hæmaturia is based in the *first event upon the* existence of cystitis On the other hand, from the transition of these tumours of the cyst he contributes to the foundation of the Virchow's irritation theory which insists that the chronic irritation may be the cause of the several tumefactions Moreover he has contributed an important opinion as to the *comparative research* of new formations, arguing by analogy from the results of the experimental research of new formations by Drs Yamakiwa and Ichikawa

(4) Moreover, based on the fact that cystitis is the cause of the chief fundamental change, he has sought for the cause of cystitis, and as the result of his researches he denies *that* spirochæta, parasite eggs act as *the causal* agent At one time he thought that a special diplococcus might perhaps be the agent but he soon returned to the opinion that more researches was required before the supposition could be confirmed At all events by means of his efforts in searching for the cause, he has been able to rule out several causal agents, or matters, one by one gradually and has succeeded in narrowing the field of research and shortening the course of producing confirmation, although he has *not succeeded in determining the actual cause*

DISCUSSION

Mr J T Edwards (United Provinces) If Dr Miyamoto had not given a summary of his *bacteriological examinations, one would have suspected from the* clinical and pathological history given, that such an outbreak in India was hæmorrhagic septicæmia, and further if this diagnosis were ruled out that it was probably caused by an intoxication of the kind that follows the ingestion of castor beans However, he mentions *that he has been able to reproduce the condition by feeding* cattle with cultures of an organism *resembling B paratyphosus B*, isolated by him from affected animals and his observations in this respect are of much interest

With reference to his second paper Outbreaks of bovine hæmaturia have been described in various parts of the world A few years ago Hadwen *investigated a some*what serious outbreak in British Columbia and the published information would seem to indicate that he believed it was caused by the ingestion of fodder plants rich in oxalic acid, which was then excreted from the system with the urine and became *deposited in* the form of insoluble oxalate crystals in the urinary bladder, there to set up chronic mechanical irritation *We have brought to our notice in India an enzootic* bovine hæmaturia by Mr Kerr, Veterinary Adviser to the Government of Bengal, among cattle

in the Himalayan foothills near the Darjeeling district Examination of material forwarded to us at Muktesar revealed merely a hæmorrhagic inflammation of the mucous membrane of the urinary bladder without indication of any specific bacterial infection Mr Kerr believed the outbreak to have been caused by the ingestion of certain food substances actually bamboo leaves, the effects were particularly noticeable among imported cattle

The progressive development of the condition reported by Dr Miyamoto, with continued irritation into neoplasm formation is of exceptional interest and if his specimens reveal genuine malignant new growth merits close attention

Mr I Krishnamurti Ayyar (Madras) I would like to be enlightened on this point —

Whether agglutination tests of the serum from natural cases of infection with gastro enteritis hæmorrhagica were conducted with the organism isolated and if so with what result

With reference to his paper on urocystitis hæmorrhagica if it is due to irritation, the nature of the changes that may be set up in the bladder may show the character of a papilloma On the other hand, if the changes set up are malignant in character, the growths which may appear to be benign in the early condition are not really so but only the beginning of malignancy for if one examines these growths carefully one may find at the bases of the projections the epithelium invading the underlying tissue in the form of strands and in more advanced cases ulceration setting in invading and destroying the papillomatous growth In those cases in which no growths are observed the wall may be infiltrated and invaded widely Dr Miyamoto in his paper describes such complex conditions as Both carcinomatous and sarcomatous hypertrophy,' ' Superficially carcinoma deeper part large spindle celled sarcoma and ulcus formation ' etc in one and the same growth It is quite possible for different conditions such as either papilloma carcinoma round celled sarcoma or spindle celled sarcoma to occur, but such complex conditions as described by Dr Miyamoto occurring in one and the same growth are very interesting and require elucidation

Major Tosinobu Miyamoto (Formosa) In reply to Dr Krishnamurti s question as to the agglutination test The serum from several natural cases which were in the convalescent stage were tested with the organisms isolated with very definite positive results and they were also tested with several kinds of organisms which belong to typhus group but without such remarkable results as the former I did not bring the note of this caption on the journey with me If wanted I will send the detail as to the test after my return to Formosa In the abstracts the agglutination tests were omitted

STRONGYLOIDOSIS INTESTINALIS IN THE FARROW OF FORMOSA

BY

MAJOR TOSINOBU MIYAMOTO

Veterinary Research Officer Government Research Institute Formosa

FORMOSA is one of the most intensive pig rearing lands in the world the annual production (1925) totalling 1 450 000, making the average of 3 61 per farmhouse

Pigs form the leading flesh food of the Formosans and by official protection and encouragement the Government seeks to improve the stock and raise the standard of breeding and development Expert and efficient bacteriological attention is concentrated on pigs' diseases and defects Recent research has sought a remedy for the ailment familiarly known as 'white discharge' or white diarrhœa' in the sucklings Formosan pig raisers have for many years endeavoured to detect a cure

ÆTIOLOGY

Cause —Parasites of the genus *Strongyloides* (familiar in many parts of the world in humans domestic animals and others)

Speciation of the group is not easy

Description —The writer in autopsy on piglings has met with two species of these Strongyloides in the small intestine

(1) The parasitic generation of *S suis* (? Lutz 1885) or *S longus* The parathenogenetic female

(2) That of *S westeri Ihle* (1917) *S suis* having been fully described by several authorities (especially in the recent papers of Asa C *Chandler and T H Sandground*) only a few need be mentioned here

1 Specimen from example No 30

Total body length	3 7	mm
Œsophagus	1 0	„
Between the end of œsophagus and vulva	1 5	
Between the vulva and anus	1 0	
Tail or between anus and tip of tail	0 2	
Ratio of œso to total body length	1 0 3 7	

2 Specimen from example No 26

Total body length	3 96
Œsophagus	0 8?

Tail	0·1 mm
Body breadth at the proximity of vulva	0·051
Body breadth at the middle part of œsophagus	0·82 "
3 Another specimen from the same example	
Total body length	2·56
Œsophagus	0·81
Between the end of œso and the loop of anterior uterus	0·0021
Between the end of œso and vulva	1·00 "
From vulva to the tip of tail	0·75
Total	0·045
From vulva to the loop of anterior uterus	0·08
From vulva to the loop of posterior uterus	0·08
Body breadth at the top of body	0·0192
Body breadth at the proximity of vulva	0·0487 "
Whole length of uterus stripped out of the body	1·96

Under low magnification the cuticula appears almost smooth

The shape of the tail resembles that of the type in the *S papillosus* group The uteri are disposed anteriorly and posteriorly of the vulva as origin The anterior specimen forms a loop near the end of the œsophagus and after two or three twists turns back terminating in a blind tube The posterior example forms directly a hair pin bend closely above the anus, being thus once or only half twisted As seen above the proportion between size and length varies possibly this development is influenced by time and environment

The adoption of the classification S westeri suggested itself to the writer on account of the large size revealed in the autopsies undertaken by him The body length reaches 9 mm or more but in general shape and uterine disposition the specimens may perhaps be considered as highly developed *S suis* and not separately classified as a distinct group

Both the above suggested groups are whitish in colour and the specimens are so large and distinct as to clearly indicate thin white filaments to the naked eye in the contents of a scraping of the mucosa from the small intestine when spread on the surface of the object glass The thread like worm is fairly active moving itself into curves rings and twists constantly varying its contortions the head and tail moving with special freedom in all three dimensions as can be readily observed under the microscope

The distribution of these parasites in the intestine is almost homogeneous throughout every part of the small intestine but sometimes they swarm at the duodenum and anterior section of the jejunum as indicated in example No 22, in which case the writer divided the small intestine into about twenty sections of equal length commencing at the origin of the duodenum working backwards each several section being minutely examined

The worms are not only present in the contents of the intestine but they also lodge fairly deeply in the mucosa between the villi In the large intestine, worms do not appear to exist only a fragment of a dead worm being occasionally detected therein

In the uterus many developed ova are in line along the longitudinal axis near the vulva the line of unripe ova extending into the remote section

The Ova —Shape	oval	Average length 49 microns (47 to 56 microns)
		Average width 37 (28 to 31)
Shell		Thin but strong
Contents		Already differentiated into small larvæ which actively twist and wriggle into S' bends

These eggs while in the uterus are packed in the clear mucilaginous membrane secreted by the uterine wall and are lodged in a row, being in due course exuded per vagina in the slough The slough carries as many as 8 12 14 18 and 23 eggs at a time which are also found in an abundant mass in the *contents of the* small intestine or in deeper sections of the villi of mucosa as observed in the cut preparations In due course the slough ruptures probably through mechanical action such as peristalsis the vigorous motion of the larvæ or such chemical influences as the digestive juices and the strings or rows of eggs are distributed

Therefore except where the parasite or mother worm deposits the eggs in strings (as in the upper part of the intestine) only isolated or dispersed ova are generally found

Near the terminus of the ileum such separated eggs are usually readily discovered In the large intestine and in the fæces only detached individual eggs are found

As a rule they do not hatch before leaving the body of the host but on rare occasions a few larvæ are found mixed with many eggs in the intestinal contents near the anus

In summer the eggs in the fæces usually hatch within 24 hours of defecation while the larvæ hatched before expulsion are thin especially anteriorly the œsophagus is short and the posterior end tapers evenly to the tail tip which is acutely pointed

These free larvæ, rhabditiform develop and the small and large larvæ are seen swarming in the stale fæces mingled with a few unhatched unfertile eggs The larvæ are also detected in the fæces of the mother sow The filariform larvæ are met with during inspection of the filth in the swine styes of several districts in Formosa

The resistance of the ova to dryness is fairly strong Material conveyed long distances and drying in the course of time may contain ova and larvæ which have become shrunken and motionless seemingly dead but the addition of moisture gradually revives them and they become turgescent and animated

The rhabditis larvæ are also fairly resistant to dryness but cannot stand chemical agents such as even a weak solution of formalin

Pathogenicity

It would be difficult to find an adult pig so seriously infected with the parasites of the strongyloid nematodes as to appear diseased or actually unhealthy On the other hand the parasite usually disappears apparently on the attainment of the pig to adult age being eliminated naturally on the animal surviving the suckling age so that piglings which were infected mature into animals free from the parasites giving negative results microscopically for the mother worm in the intestine and the eggs and larvæ in the fæces The full grown moderately infected animal may show no visible indication of nematodes but may constitute a carrier

Pig raisers and breeders must be warned against this as several otherwise healthy sows have infected their litters as has been actually observed Moreover it has been indicated that the adult animals show a good resistance to these parasites while the young ones are readily infected Thus the detection of the Strongyloides for a long time escaped the notice of the Formosan veterinarians who had directed their inspection chiefly to full grown pigs which came to the slaughter house or to other mature or semi adult animals which had come under their therapeutic investigations Any malady of the sucklings was generally considered as trivial and was therefore neglected or the ailing piglings were weeded out in early stages As is well known in other parts of the world the young of domestic animals (calves colts lambs and piglings) when infected with nematodes become ailing fail to develop and often die In Formosa and probably in other parts of Japan there prevails enteritis caused by Strongyloides in the form of white fæces or diarrhœa but there are not sufficient grounds to state that all the so called white diarrhœas are caused by this parasite or that Strongyloides enteritis covers all the cases of white fæces or diarrhœa There may be not infrequently non parasitic causes of white fæces or diarrhœa which give a negative result as regards worms or eggs in the microscopical inspection of the intestinal content or fæces Such conditions may appear in cases in which the intestinal digestion is disturbed by several influences such as too much sucking over stuffing dyspepsia certain forms of enteritis etc In such cases however the ailment is usually very light and is readily cured by a short course of rational feeding Other parasites may also contribute to cause a similar disturbance of intestinal digestion such as *Ascaris lumbricoides* but in the Ascaris infection the first appearance of the fæces discoloration is later and incomplete while in the case of Strongyloides infection the change takes place very soon after birth on account of early infection or intrauterine infection

On the other hand white fæces does not invariably appear in all cases of Strongyloides infection in young animals

Exceptions may arise in the degree of disturbance of the digestive function or in other words it depends upon the number and vigour of the parasites While therefore the worms are so few or weak that they may not be sufficiently numerous or strong to disturb the function of the intestine or to change the colour of the fæces

In the uterus many developed ova are in line along the longitudinal axis, near the vulva, the line of unripe ova extending into the remote section

The Ova —Shape	oval	Average length 49 microns (47 to 56 microns)
		Average width 37 " (28 to 34 ")
Shell		Thin, but strong
Contents		Already differentiated into small larvæ which actively twist and wriggle into 'S' bends

These eggs, while in the uterus are packed in the clear mucilaginous membrane secreted by the uterine wall, and are lodged in a row, being in due course exuded per vagina in the slough The slough carries as many as 8, 12, 14, 18 and 23 eggs at a time, which are also found in an abundant mass in the contents of the small intestine, or in deeper sections of the villi of mucosa as observed in the cut preparations In due course the slough ruptures, probably through mechanical action, such as peristalsis the vigorous motion of the larvæ, or such chemical influences as the digestive juices, and the strings or rows of eggs are distributed

Therefore except where the parasite or mother worm deposits the eggs in strings (as in the upper part of the intestine) only isolated or dispersed ova are generally found

Near the terminus of the *ileum* such separated eggs are usually readily discovered In the large intestine, and in the fæces, only detached, individual eggs are found

As a rule they do not hatch before leaving the body of the host, but on rare occasions a few larvæ are found, mixed with many eggs in the intestinal contents near the anus

In summer the eggs in the fæces usually hatch within 24 hours of defecation, while the larvæ, hatched before expulsion are thin especially anteriorly, the œsophagus is short and the posterior end tapers evenly to the tail tip which is acutely pointed

These free larvæ, rhabditiform, develop, and the small and large larvæ are seen swarming in the stale fæces mingled with a few unhatched, unfertile eggs The larvæ are also detected in the fæces of the mother sow The filariform larvæ are met with during inspection of the filth in the swine styes of several districts in Formosa

The resistance of the ova to dryness is fairly strong Material conveyed long distances and drying in the course of time, may contain ova and larvæ which have become shrunken and motionless seemingly dead, but the addition of moisture gradually revives them, and they become turgescent and animated

The rhabditis larvæ are also fairly resistant to dryness, but cannot stand chemical agents, such as even a weak solution of formalin

PATHOGENICITY

It would be difficult to find an adult pig so seriously infected with the parasites of the strongyloid nematodes as to appear diseased or actually unhealthy On the other hand the parasite usually disappears apparently on the attainment of the pig to adult age being eliminated naturally on the animal surviving the suckling age so that piglings which were infected mature into animals free from the parasites giving negative results microscopically for the mother worm in the intestine and the eggs and larvae in the fæces The full grown moderately infected animal may show no visible indication of nematodes but may constitute a carrier

Pig raisers and breeders must be warned against this as several otherwise healthy sows have infected their litters as has been actually observed Moreover it has been indicated that the adult animals show a good resistance to these parasites while the young ones are readily infected Thus the detection of the Strongyloides for a long time escaped the notice of the Formosan veterinarians who had directed their inspection chiefly to full grown pigs which came to the slaughter house or to other mature or semi adult animals which had come under their therapeutic investigations Any malady of the sucklings was generally considered as trivial and was therefore neglected or the ailing piglings were weeded out in early stages As is well known in other parts of the world the young of domestic animals (calves colts lambs and piglings) when infected with nematodes become ailing fail to develop and often die In Formosa and probably in other parts of Japan there prevails enteritis caused by Strongyloides in the form of white fæces or diarrhœa but there are not sufficient grounds to state that all the so called white diarrhœas are caused by this parasite or that Strongyloides enteritis covers all the cases of white fæces or diarrhœa There may be not infrequently non parasitic causes of white fæces or diarrhœa which give a negative result as regards worms or eggs in the microscopical inspection of the intestinal content or fæces Such conditions may appear in cases in which the intestinal digestion is disturbed by several influences such as too much sucking over stuffing dyspepsia certain forms of enteritis etc In such cases however the ailment is usually very light and is readily cured by a short course of rational feeding Other parasites may also contribute to cause a similar disturbance of intestinal digestion such as *Ascaris lu nbr coides* but in the Ascaris infection the first appearance of the fæces discoloration is later and incomplete while in the case of Strongyloides infection the change takes place very soon after birth on account of early infection or intrauterine infection

On the other hand white fæces does not invariably appear in all cases of Strongyloides infection in young animals

Exceptions may arise in the degree of disturbance of the digestive function or in other words it depends upon the number and vigour of the parasites While therefore the worms are so few or weak that they may not be sufficiently numerous or strong to disturb the function of the intestine or to change the colour of the fæces

many eggs may even occasionally be detected in normally yellowish or brownish fæces

Origin of White Fæces or White Diarrhœa

Coagulated and incompletely digested mother milk is the cause or origin of the white fæces In colour it is milky white, sometimes slightly yellowish orange through the influence of bile and the creamy mass is sticky, fairly hard viscous with minute forms sometimes spherical sometimes like rolled sticks of chalk The indigestion is caused by the invasion of Strongyloides in the small intestine If the quantity of water contained in the fæces is excessive, the consistency decreases, and becoming semi liquid, it stains the surroundings of the anus and also the stall bed proving that the intestinal absorption has diminished, or indicating the complication of colitis catarrhalis White fæces or white diarrhœa discharges usually bear a characteristic odour as difficult to remove by washing as ointment Towards the end of the milking period when the diseased animals begin to eat some food the fæces then gradually change in colour, depending on the food and obstinate diarrhœa develops containing a large number of the Strongyloides eggs Later watery diarrhœa, mixed with mucous and blood, indicates the complicated condition of severe cæco colitis in most cases with Balantidium infection in the large intestine This produces the stage of inflammation of the small intestine caused by Strongyloides and inflammation of the large intestine resulting from Balantidium

Occasionally the diseased or healthy young pigs running in grassy pasture, may chew, eat or suck some vegetable roots with soil adhering or in some other way nibble soil which acts as an allotriophagia As a result of these incidents the white fæces sometimes diminishes and the eggs in the fæces become fewer and a natural remedy appears to be thus introduced through animal instinct

Density of Infection or Enumeration of the Eggs and Worms in the Host Intestine

Taking as an example of moderate infection, let me refer to recorded case No 52

Breed Pure Berkshire Male

Age .75 days

Dead and dissected on 26th June, 1927

By test inspection it was confirmed that the distribution of parasites was almost homogeneous throughout the whole length of the small intestine, the length thereof being 760 cm while that of the large intestine, cæcum and colon was 96 cm

Between the origin of the duodenum and the terminus of the ilium, seven pieces of one cm each in length were cut off these sections being about one metre apart The contents of each segment were completely dropped on slide glasses forming nine or ten spots of about equal size, and each spot was spread to a thin layer to facilitate the count of the parasites it contained

The sum f the parasites in each individual spot therefore indicates the number to be found in a cm section of the corresponding portion of the small intestine Also the average number of the parasites contained in the seven separate sections indicates the average content of each cm in the entire length of the small intestine

Resulting from this method of deduction we find

Average content of a segment of 1 cm length

Worms 2 to 5 average being 4

Eggs 300 to 600 average being 400

or of the entire length of the small intestine

Worms actual say 1 500 to 3 750 (average 3 000) 750×4

Eggs actual say 225 000 to 375 000 (average 300 000) 750 ×400

In the case of the large intestine the content per one cm is much more quantitative than in the small intestine and the eggs per sectional cm of the large intestine would therefore be more numerous

A proportion of the worms or eggs may have remained deeply adhering in the *mucosa and have therefore escaped the above enumeration or calculation*

Occurrence

The disease occurs epizootically in the majority of the members of a litter or in all of them as a family or stall disease

The outbreak is much intensive in the stall and much rarer in the free field feeding litters It is also more severe in the newly imported high breeds than in the native stock because there are many opportunities for invasion per os or per derma in the stall where numerous farrow lying with their mother sow suck and sleep in mass the sow being occasionally the carrier of the causal agent The disease prevails throughout the year but especially during the hot and wet seasons

Natural Infection —Corresponding to the infection which commences soon after birth indigestion sets in through the inflammation of the small intestine caused by the parasites and malnutrition also by insufficient absorption of chylus Moreover the lesion of the mucosa caused by the mechanical action and decomposition products of parasites will introduce the invasion of some species of micro organisms pathogene The meconium is normally bluish black of the size of onion seeds or slightly larger grains In the natural infection the abnormal colour of the fæces is sometimes detected as early as the third day after birth but is more generally met with after the seventh day The abnormal colour of the fæces is the sign of intestinal disorder and indicates the existence of the parasites

In the early cases such as those detected on the third day after birth it is reasonable to presume that probably placental infection already occurred in the fœtal stage

In fact I have found in autopsy the full grown parasites with ripe eggs and many free eggs in the intestinal contents of piglings only three or four days old,

as in Example No 18 and in the meconium mixture also exuded from specimens already dead or discarded as not worth rearing Abnormal fæces are to be seen, at first as solid brown or blackish small bean sized grains The solidity and colour gradually decrease soon changing to a semi liquid white Sometimes the normal fæces develops directly into thin yellowish matter and after two or three days (or more) turns almost completely white

In moderate cases improvement may occur after five or six days, but in more severe instances the ailment continues for a week or longer, in which event the prognosis is doubtful in most cases and death results after a month or so If the obstinate diarrhœa continues for a month or longer, the liquid fæces changes latterly in colour to a greyish yellow or dark grey It is moreover mixed with mucous or blood The increase of body weight, becomes slow, or ceases entirely later showing a decrease At the same time emaciation and depression set in, usually complicated with other diseases and terminating in death

Anatomical Changes

The details of each case are here omitted but are the same as given in Table I The autopsies of young pigs have been collected and recorded covering eighty or more cases the greater number relating to white diarrhœa the remaining few to the anamnesis of under development depression, cough etc

TABLE I

Number	Breed	Age in days	Termination	Chief symptom or changes	Parasites
1	B	75	Killed	White diarrhœa invaginatio ilei	A S
2	B H	60	Death	White d	S
3	B	50	Killed	Pneumonia, d	b
4	B H	50		Hepatitis, d	b S
5		80		White d under developed	S
6		101	Death	Invaginatio ilei	S W
7		65	,		S
8		100	Killed	Colitis diphtheritica d	S W
9		98	,	, ,,	S b
10		98		Invaginatio ilei	S W
11	N	90	,	Hog cholera	A S

TABLE I—*contd*

Number	Breed.	Age in days	Termi nat on	Chief symptom or changes	Paras tes
12	B	40	Killed	Wh te d under developed	S
13	B H	30			S
14		40		"	S
15			Death	Pneumonia acuta	Negat ve
16	B	82		Enteritis hæmorrhag ca	S
17		103		Enter tis hæmorrhag ca d phther t ca	S W
18		5	Killed	Enteritis hæmorrhagica	S
19	B H			Colitis diphtherit ca	S
°0		85	Death		S W
21	B	95		Dilatat on of small intestine colit s diphtheri tica	b S W
22		1°3		White d	S
23	B H	60	Killed	D under developed	S b
24	B	62			S b
°5		74	Death	Perforat on of intestinal wall by ascar s	A S
°6	B H	7°		D	S W
27	B	89		D	S W
°8		59		D	S W
29	B H	33		White d.	S W
30	B	100		D	S
31		135		D under developed	S
3°	B H			Dilatat on of small ntest ne d	S
33		10			S
34		2		Hairless naked born	S
35		°1	Killed	Under developed	Negat ve
36		169	Death	Invaginatio ilei	S
37		19		Pneumonia	Negat ve
38	B	31		Wounded	S
39		1		No white fæces	Negat ve

as in Example No 18, and in the meconium mixture also exuded from specimens already dead or discarded as not worth rearing Abnormal fæces are to be seen, at first as solid brown or blackish small bean sized grains The solidity and colour gradually decrease soon changing to a semi liquid white Sometimes the normal fæces develops directly into thin yellowish matter, and after two or three days (or more) turns almost completely white

In moderate cases improvement may occur after five or six days, but in more severe instances the ailment continues for a week or longer, in which event the prognosis is doubtful in most cases and death results after a month or so If the obstinate diarrhœa continues for a month or longer, the liquid fæces changes latterly in colour to a greyish yellow or dark grey It is moreover mixed with mucous or blood The increase of body weight, becomes slow, or ceases entirely later showing a decrease At the same time emaciation and depression set in, usually complicated with other diseases and terminating in death

Anatomical Changes

The details of each case are here omitted, but are the same as given in Table I The autopsies of young pigs have been collected and recorded covering eighty or more cases the greater number relating to white diarrhœa, the remaining few to the anamnesis of under development depression, cough, etc

TABLE I

Number	Breed	Age in days	Termination	Chief symptom or changes	Parasites
1	B	75	Killed	White diarrhœa invaginatio ilei	A S
2	B H	60	Death	White d	S
3	B	50	Killed	Pneumonia d	b
4	B H	50		Hepatitis, d.	b S
5		80		White d, under developed	S
6	,	101	Death	Invaginatio ilei	S W
7	,	65	,,	,, ,	S
8	,	100	Killed	Colitis diphtheritica, d	S W
9	,	98	,,	,, ,,	S b
10		98	,,	Invaginatio ilei	S W
11	N	90	,	Hog cholera	A S

TABLE I—*co td*

Number	Breed	Age in days	Term nation	Chief symptom or changes	Paras tes
12	B	40	Killed	Wh te d under developed	S
13	*B H*	30			S
14		40			S
15			Death	Pneumonia acuta	Negat ve
16	B	82		Enteritis hæmorrhag ca	S
17		103		Enteritis hæmorrhagica diphther t ca	S W
18		5	Killed	Enter tis hæmorrhagica	S
19	B H			Colitis diphtherit ca	S
°0		85	Death		S W
21	B	95		Dilatat on of small intestine colit s diphther t ca	b S W
2°		123		White d	S
23	B H	60	Killed	D under developed	S b
°4	B	6°			S b
°5	,,	74	Death	Perforation of intestinal wall by as aris	A S
°6	*B H*	7°		D	S W
27	B	89		D	S W
°8		59		D	S W
29	B H	33		Wh te d	S W
30	B	100		D	S
31		135		D under developed	S
3°	B H			Dilatat on of small ntest ne d	S
33		10			S
34		2		Hairless naked born	S
35		°1	Killed	Under developed	Negat e
36		169	Death	Invaginatio ile	S
37		19		Pneumonia	Negat ve
38	B	31		Wounded	S
39		1		No wh te fæces	Negat ve

TABLE I—*contd*

Number	Breed	Age in days	Termination	Chief symptom or changes	Parasites
40	B H	210	Killed	Enteritis d	b S
41	B	70	Death	Bronchitis ac ta	Negative
4?	B H		Killed	Hog cholera	A b
43		50	Death	D	b
44	B			Under developed	A b
45	N	40		Hepatitis constipation	S
46	B H	?30			S
47		93		Allotriophag a l	S
48				D under developed	S
49	B	50	Death		S
?0	B H	?0			S
51		114		Hog cholera	S A
5?	B	7?		Colitis d phther t ca	S b
?3	B H	1?0	Survive	Under d velope l	b
54		9?	Death	D	b
55	B	6?	Survive	Under developed	S
56		3	Death	Congestion of k dney	Negative
57		12		Poor appetite	S
58		38		Invaginatio ile	S
59		41		Colitis d phther t a	S
60		14			S
61		16		D	S
6?	B	7		Dilatat o l i	S
63		15		Poor appetite	S
64		100		D	S
65		?7		Allotriopha a	S
66					S W
67		?(			S

TABLE I—concl l

Number	Breed	Age in days	Termi nat on	Chief symptom or hanges	Para tes
68	B	°8	Death	Dyspeps a	S
69		4		Swelling of spleen stomat tis	Negat ve
70		3		Pneumonia hæmorrhage	Negat ve
71		14		Poor appet te d	S
72		14		Cæcum under developed	S
73		2		Poor appet te pneumonia	S
74		15		Poor appet te	S
75		10		Invaginat o ile	S
76		12		Poor appet te d	S
77		30		Dilatat on of small intestine partly filled w th content	S
78		36		Large intestine empty	S
79		18		Under developed internal organs	S
80		90		Pleuro-pneumon a hernia inguinalis	A

Note —B —Berkshire N —Nat ve pig B H —Berkshire hybrid b —Balant d um
S —Strongylo des W —Whip worm A —Ascaris D —D arrhœa

Cha ges —Malnutrition general anæmia under development of the digestive organs scanty contents of the digestive tube and parasites present tl erein

Stomach —Filled or empty the quantity of the contents variable according to the c ndition of the appetite before death The contents are chiefly coagulated milk in the sucklings sometimes n ixed with otl er materials sucl as straw chips soil hair etc

Small intestine —Generally dilated as if paralysed Sometu ies partly or even entirely filled with milk coagula as in the stomach this continuing downwards

instead of being empty as in the case of healthy animals The dilatation is limited locally, long or short and many short diverticula resembling dilated parts are found throughout the whole length of the small intestine The wall of these dilated parts is very thin, the mucosa and sub mucosa being extremely distended

The *dilated parts contain* liquid in which worms and eggs are floating, invaginatio jejuni et ilei are repeatedly met with in autopsy (about 10 per cent) Sometimes one or more parts of the small intestine in the same animals are invaginated, the length of such parts varying from 2 or 3 cm to 4 or 5 cm The cause of the invagination may be attributed partially at least, to the disturbance of the intestinal function caused by the parasitic worms

Congestion and hæmorrhage in the mucosa of the small intestine are met with in about 25 per cent of the cases The hæmorrhage occurs in scattered points or is diffused The contents also become reddish and the mucosa falls off very readily

Large intestine—Ulcers and productive and purulent inflammations in the mucosa are repeatedly met with We also occasionally find congestion, hæmorrhage or nodule formation in the mucosa, and dilatations or local constrictions of the tube These ulcers, nodules and productive or diphtheritic changes in the *mucosa are probably caused by the complication of other ciliatæ amœba* whip worm, etc

Other organs—*Ren cysticus Hydrops renalis cysticus* are met with comparatively often, being possibly a congenital anomaly not directly related to the parasites Pneumonia catarrhalis, corresponding to the symptoms occurring during life, is also occasionally detected

The chief internal parasites, with the exception of the Strongyloides, are *Ascaris lumbricoides* in the small intestine and whip worm and *Balantidium coli* in the large intestine

Under development of sucklings or young pigs—The under development, deficient body weight small size and lack of vigour are indications of the abnormal state of the health of the animals A litter composed of the several individuals from one sow is often altogether inferior, in other cases one or two of the litter farrowed from a sow on the same day, may be comparatively inferior in all points This inequality or inferiority is mainly congenital, and is caused by a certain deficiency in the internal organs

On the other hand, this deficiency may be acquired and developed by the *invasion of Strongyloides*

The measure of deficiency or under development may be ascertained usually by the weight of the body or more minutely by that of the internal organs I have noticed *in numerous autopsies that* the range of difference of magnitude of the organs (thoracic or abdominal) of different cases is very wide, and relative changes are not often parallel in each case I will, therefore give in detail the length of the intestines *of the cases as the representative organs in* Table II which covers twenty cases

TABLE II

Number of animals	Age of animal by days	Length of small intestine	Length of large intestine	Termination	Parasites	Diagnosis etc
1	5	98 cm	60 cm	Killed	S	White diarrhœa
	21	375	34		Negative	
3	30	303	33		S	
4	40	991	121		S	
5	40	1190	115		S	
6	50	513	6		S P	
7	50	535	90 8		B	
8	60	870	230		S	
9	6	742	133	"	S B	
0	65	1030	130		S	
11	75	363	59		A S	
12	75	750	96	Death.	S B	
13	80	810	156	Killed	S	
14	82	1082	191	Death	S	
15	85	636	109	Killed	S	
16	90	1191	191		A S	
17	98	733	155		W S	
18	98	970	165		W S	"
19	100	630	100		W S	
20	803	994	154	Death	W S	
21	1 3	930	18		S	
2	210	1435	295	Killed	B S	

Note —S —Strongyloides B Balantidium A Ascaris W —Whip worm
The length of œsophagus of No 9 16 cm
The intestine of No 11 lr ely sl unk by formalin dip

CONCLUSION

Formosa is recognized as one of the lands infected with Strongyloides The disease locally known as white diarrhœa of the farrow is caused by this parasite This ailment is extensively prevalent but of a latent chronic character

was discovered that the virus survived in the blood when the surrounding CO_2 tension was adjusted to approximate that of the circulating blood as long as it did in control samples of blood kept under a paraffin seal The beneficial effects of the gas upon survival were manifested throughout a wide range (from 1 to 10 per cent CO in the tests)

Dilution has long been known to render the rinderpest virus rapidly inert It was found in our early tests that when the fresh infected blood was mixed with five times its bulk of diluted horse serum (1 2) or peptone solution and stored at body temperature under a thick paraffin seal, its virulence sometimes disappeared within 24 hours whereas the blood, diluted with only an equal volume of the diluent, retained its virulence for 10 days Several experiments were carried out to determine the mechanism of the rapid destruction of the virus upon dilution It was found that when the infected blood was suspended in high dilution in a well buffered solution (dilute serum peptone solution phosphate solution) adjusted so that the hydrogen ion concentration remained near that of the circulating blood, the period of survival of the virus was relatively long Again, when old stored serum the hydrogen ion concentration of which was re adjusted, by addition of acid, so that it would remain near that of the body fluids was added in replacement of the serum washed away by centrifugation from fresh virulent blood, the period of survival was considerably lengthened When added in small amounts to the re adjusted normal serum the blood retained its infectivity also for enhanced periods of time (In stored defibrinated or citrated blood, the overlying serum rapidly loses its infectivity whereas the corpuscular mass underneath retains its virulence for some time)

Contamination with certain bacteria, both ærobic and anærobic, was not found to influence greatly the longevity of the virus The addition of a small piece of fresh animal tissue (sterile rabbit kidney) to infected blood, stored otherwise in what were considered to be optimum conditions for survival, brought about a rapidly destructive effect upon the virus *It would seem that of the agencies that favour* destruction those such as are produced by autolysis of living tissues, would come into play more prominently in natural circumstances One knows that autolysis is accompanied by an increase in acid reaction, but the enhanced hydrogen ion concentration is most probably not the only change that is detrimental to the life of the virus

High dilutions would thus appear to operate unfavourably merely by allowing acceleration in the rate of destruction by the agencies present in the undiluted fluid that were already inimical to the survival of the virus

The details of this work will be published later in special memoirs, but this preliminary note is read now to indicate the class of work from which we have been able to devise the present simple system of transportation of virulent blood from Muktesar to the various places where it is required for use throughout India for the serum simultaneous inoculation of cattle A few years ago, the despatch of virus from the laboratory to reach the field operator in an active condition was a

very difficult problem, as experience had shown that when despatched in thermos flasks, it was often inert well within three days Special messengers had to be employed to convey the virus with all possible speed to the scene of use Since a change in hydrogen ion concentration, brought about by the liberation of carbon dioxide gas from the shed blood is a most important factor in the initial rapid deterioration in virulence of the blood the procedure now adopted at the laboratory is to withdraw blood at the height of the thermal reaction from infected, very highly susceptible cattle with strictly sterile precautions defibrinate it quickly and then distribute it in sterile bottles (filled up nearly completely) which are then hermetically sealed (by impregnating the cotton wool plugs with paraffin wax) as soon as possible The consignments of virus are then despatched by parcel post to the consumers who are advised telegraphically of the despatch A considerable experience at the laboratory and in the field now extending over three years has shown that virus can be despatched readily in this manner to reach consumers in an active condition for periods extending to 8 days after leaving the laboratory The original richness of the infected blood in virus is, however, a factor of considerable importance and sometimes the period of survival for this reason is likely to prove disappointing

The Implantation of the Virus on Animals other than Cattle

(*i*) *Rabbits* —*Experiments were originally undertaken in 1922 to fix the virus* upon rabbits to obtain any further knowledge upon its nature and its relationship to other groups of virus diseases that might be derived by studying its pathological action upon animal hosts other than those upon which it commonly exhibited a highly pathogenic action in natural circumstances No local reaction was induced in rabbits after massive injection of highly virulent ox blood except after intra ocular injection, when for some days peculiar changes were observed in the iris similar to those described in the disease known as specific ophthalmia in horses

Intravenous inoculation of a large quantity of virulent blood was followed, however, on the succeeding days by a scarcely perceptible rise in the temperature of the rabbit (Failure to obtain any significant rise may nevertheless, commonly occur) When blood was taken from the rabbit at the height of this curve, and injected intravenously into other rabbits, there was induced in them an enhanced thermal reaction with successive 'passage' through rabbits in this manner, the curve became very marked, so that on the second or third day there was a rise amounting to 3° F or more which then subsided to normal usually by the 5th or 6th day There were no distinct clinical symptoms manifested by the rabbits, mortality among them was rare and no distinct pathological changes could be observed in the internal tissues In this way, 'strains' of rabbit virus were kept up in the laboratory by passage at intervals of 2 days, 3 days, 4 days, and 7 days, for 14 months, after which the experiments had to be discontinued on account of the supervention of an outbreak of pasteurellosis among the experimental animals During this period the infectivity of each strain of the rabbit virus was tested upon

susceptible cattle from time to time The virus seemed to have suffered a certain degree of degradation in virulence towards cattle at an early state after implantation upon rabbits when it was measured by the readiness with which *reactions could be* blocked out by anti rinderpest serum in test cattle and the relative difficulty observed in the other cattle protected with the same serum but infected with ox virus Subsequently it did not undergo any progressive attenuation The degree of reaction in the inoculated cattle varied from types that were almost symptomless or merely febrile in character but left a powerful immunity towards infection later to those which progressed to almost the same serverity as the disease commonly observed by the injection of virulent ox blood *The modified virus never became* safe for the vaccination of cattle by itself but it had the advantage of being a 'pure virus that is it was not contaminated with any piroplasms likely to set up complicating infection in cattle if it were used for the virus injection in the serum simultaneous inoculation It seemed from some experiments that rabbits were capable of retaining the virus for a long time (2 months) in minute quantities in their bodies after the acute febrile reaction to the infection had subsided

(ii) *Small Ruminants* —Outbreaks of natural rinderpest *among goats* and sheep have occasionally been reported but it has been a matter of great difficulty to maintain the virus by passage in these animals at the laboratory We had found that after inoculation of large quantities of virus of bovine origin intravenously, it persisted in minute quantities for some time afterwards in the blood stream of the smaller animals though its appearance as disclosed by test inoculations of blood into susceptible cattle, was irregular (It is of interest to recall that Ruppert in *German East Africa some years ago claimed to have demonstrated a persistence* of the virus in the blood of sheep for 6 months) It had been found impossible to propagate the virus in the smaller animals however, by passage, there was therefore, no exaltation of virulence after the first inoculation, and probably the failure at a second passage could be accounted for by assuming that the virus was present in blood of the first sheep only in very small numbers and the amount present in the sub inoculated blood was too small to establish itself in the system of the second sheep

It came to our notice that Beller, in Germany, had found that, when cultures of the virus of contagious bovine pleuro-pneumonia were injected into sheep, examination of the blood serum (by the complement test) indicated that the virus survived in some obscure location in the bodies of the infected sheep *for a long time afterwards* Pregnant sheep subjected to infection in this way, sometimes aborted after some time and bacteriological and pathological examinations revealed the fact that the virus had become established in the tissues of some of the fœtuses there to set up lesions closely resembling those of the natural disease in cattle although no trace of infection could be discovered in the maternal tissues

These experiments were carefully repeated at Muktesar upon pregnant sheep by inoculating a series with *rinderpest virus of bovine origin* At prescribed intervals subsequently, the sheep were scarified and the maternal blood and fœtal juices and

tissues tested separately for virulence by inoculation into susceptible hill bulls The virus was found to persist irregularly for some weeks after infection in the maternal blood but no evidence was obtained of its establishment at any time in the fœtal tissues The fœtal envelopes would thus have acted as an effective barrier against the penetration of the virus in its feeble state of specific virulence for the sheep into the fœtal circulation

It then suggested itself that it would be worth trying to ascertain the effects of introducing the bovine virus by careful surgical operation on the fœtal side of the fœtal envelopes in a pregnant small ruminant Goats were selected for this purpose After three days the virus was then found to circulate freely in the maternal blood and the mother showed a distinct temperature reaction Blood now taken from the mother readily infected other goats by ordinary subcutaneous inoculation and thereafter the virus was easily propagated in goats by successive inoculation of small quantities of blood and attained gradually a high degree of virulence for these animals Thus until the 9th passage three goats died and 11 recovered from the 10th to the 19th passage 13 goats died and four recovered The virus has now been maintained in goats at Muktesar for a year both by direct passage and by alternate passage through goats and bulls The goat virus has also been found to possess a high degree of natural virulence for sheep It retains its affinity for the small ruminants even though after the initial implantation it is maintained by direct passage in bovines It spreads from goat to goat by natural contact during cohabitation The footing given to the virus originally by inserting it into the presumably less resistant fœtal tissues would have enabled it to gain the necessary increase in specific pathogenicity to thrive readily in the tissues of the smaller ruminants

The clinical symptoms and tissue changes observed in the infected goats might readily be mistaken for those of some other disease The morbid changes are largely and often principally pneumonic and not almost entirely alimentary as in bovines It is now suspected that some outbreaks of so called pleuro pneumonia in sheep and goats in India are in reality rinderpest The lungs in both affections exhibit the same histological changes and also become infected early with large numbers of certain minute bacterial micro organisms The symptoms in the artificial rinderpest in goats are striking about the third day or even earlier the animal stands with an anxious expression arched back rough upstanding coat and an increased breathing rate The temperature becomes very high (103 to 107° F) on the fourth day and the temperature curve is often a prolonged one Marked nasal discharge is common and later there is sometimes much incrustation with sloughing of the nostrils Diarrhœa and rapid wasting are marked later symptoms Not infrequently the animal suddenly collapses early during the febrile reaction

The blood of the artificially infected goats is virulent as early as the second day after subcutaneous infection and by the fourth day it has often reached a very high degree of virulence We have tested it by inoculation into susceptible hill bulls in quantities varying from 1 100 c c to 1 10 000 c c and have frequently found the

ubiquitously, carriers of piroplasms, and so the results of any experimental tests are rendered invalid I recall, however, that it is stated in the literature that *P bigeminum* will remain alive in blood stored in the ice chest for about 50 days, and there is much reason to believe that the period of survival of the piroplasm ordinarily in blood, will not differ greatly from that of the rinderpest virus itself Hence, I am afraid that any attempts at differential action on the two viruses by storage would prove ineffectual, especially as the period of survival of the rinderpest virus itself may vary considerably, depending largely, I believe, upon its original richness in the blood

Col Williams asks me to describe at greater length the symptoms observed in goats affected with rinderpest (description given), and recounts the clinical appearances of an outbreak of pleuro pneumonia among sheep in Waziristan As in that outbreak, the occurrence of a marked nasal discharge is often a very prominent symptom in the artificial rinderpest of goats The nature of the infection in the goats in the course of an outbreak might be decided by inoculating some blood from an affected goat at the height of the febrile attack, that is early during the course of the disease, into a susceptible ox and observing whether symptoms of rinderpest were induced in it

RINDERPEST SOME POINTS IN IMMUNITY

BY

J T EDWARDS

Imperial Institute of Veterinary Research Muktesar

THE following notes are based upon the results of extensive observations upon certain phenomena in the immunity of cattle against rinderpest made at the Muktesar laboratory during the last six years

The Immunity developed by Young Calves

It is obviously economical to apply the serum simultaneous method of immunization to cattle at as early an age as possible provided the method can be adopted with equal safety upon young as on more mature animals and the resulting immunity is of the desired quality in degree of solidity and duration There has been in cattle also a technical reason to recommend the desirability of intervening at an early age very young cattle possess a high degree of resistance towards the development of morbidity following upon infection with *Babesia (Piroplasma) bigemina* which resistance diminishes gradually with advancing age the inadvertant introduction of the piroplasms with the blood used for the virus injection would therefore be less likely to lead to untoward complications in young animals

The results of our work at Muktesar indicate that very young calves in healthy condition can be safely immunized by the serum simultaneous method Calves specially reared in our experimental dairy herd together with some calves kindly lent to us by the military dairies have been used for this purpose their age varying from a few days to about six months The number tested already has been 64 and only one has succumbed which was in weak condition and was already apparently suffering from the bacterial disease which led to its death The calves were some of indigenous breeds and others were of cross European origin The type of reaction through which these calves passed was almost invariably of the 'blocked out or symptomless kind

The immunity of the calves has been tested by inoculation with highly virulent blood of groups at various intervals after immunization The results have shown that the immunity remains a solid one for two years after the serum simultaneous inoculation Thereafter it commences to wane and as the interval approaches three years the cattle react very distinctly to the test inoculation

The Egyptian records would appear to indicate that the immunity following upon the serum simultaneous inoculation as disclosed by actual tests on the immunized cattle lasts in a solid form for ten years at least *Our results would* seem at first sight not to bear out that experience There is available in the literature some testimony pointing to the unsatisfactory nature of the immunity developed by young calves However much further research work needs to be executed upon this problem to ascertain, (i) whether cattle in India generally develop a shorter immunity after protection by the serum simultaneous method than has hitherto been accepted from the observations made in other countries *(ii) whether the duration of immunity bears any relationship to the degree of* reaction that arises in the course of the immunization , (iii) whether age is a factor of much significance in the durability of the immunity

In actual practice it should prove relatively easy to ' freshen ' up the immunity in animals that have been subjected to the protective treatment on establishments or in localities where the method was undertaken systematically In these circum stances it is presumed that operations would be carried out once a year or once *every two years to apply the method of serum simultaneous inoculation to all stock* that had been recruited to the establishment or locality since the date of the antecedent operations On each such occasion virulent blood would be available and a minute quantity could be injected—alone without simultaneous serum injection—at negligible cost to all cattle that had been immunized at preceding operations If the indications of the calf experiments were then found to apply generally it would suffice to repeat inoculation with virus alone once every two *years or so in the animals immunized originally by the serum simultaneous method* that is before the immunity had worn off to such a degree that the animals needed further protection with anti serum to withstand the effects of a virus injection

It has been stated in the preceding paper that the procedure now recommended for local manufacture of virus—by propagation of clean ' virus which is adapted to goats and can be obtained readily from the laboratory for the injection of the goats—renders it easy for the operator to obtain supplies of suitable virus at will

It will probably be found in practice also that the age at which cattle can be most suitably immunized will be from one year to 18 months

The Development of Immunity in Relation to Climate

It had been implied in directions issued a few years ago from the laboratory that the serum simultaneous inoculation ought to be applied to cattle on the plains only during the colder months of the year To ascertain whether there were any real grounds for this restriction a brew of highly potent anti serum was stocked to be used for testing hill bulls simultaneously at intervals of about three months throughout a period of two years at Muktesar (in the Himalayan foothills altitude *7 500 feet) and Bareilly (on the plains)* The dates of testing were chosen in this manner so as to obtain knowledge upon the limiting influences of a tropical ' or

'sub tropical' climate on the one hand and of a 'temperate' climate on the other hand upon the resistance of the animal body when it was given a certain degree of protection by the serum The test performed at each of the two centres on each occasion conformed with the usual standard test for a rinderpest anti serum adopted at Muktesar that is there were in each test six hill bulls injected with virus and serum (two at 90 c cs two at 60 c cs and two at 30 c cs per 600 lbs body weight) and two controls injected with virus alone

The serum in the course of these tests manifested no marked differences in protective action at the two centres Indeed in one of the later simultaneous tests, conducted during the hottest time of the year the potency appeared to be higher at Bareilly than at Muktesar This observation may be readily explained in the light of the investigations of recent workers upon climate in relation to the health of human beings wherefrom it would seem that the basal metabolism of inmates of confined dwellings in temperate countries may be actually lower than that of residents in the tropics The animals in the simultaneous tests in question were housed at Muktesar in substantially built closed stone sheds designed to protect against the adverse effects of very cold weather whereas the Bareilly animals received shelter only in lightly built straw *chhuppers* within which the aerial envelope or cushion that would otherwise tend to develop around them would be rapidly displaced

Incidentally the tests showed also that a serum of high potency stored in the temperate conditions of Muktesar maintains its protective properties for two years although towards the end of this period these properties were diminishing to a noteworthy degree

The practical outcome of the tests would be that there is no additional danger in undertaking the serum simultaneous inoculation in the hot weather, provided the animals subjected to it are allowed to remain in conditions of environment such as ordinarily affect least their state of well being

The Degree of Reaction required (i) for the Production of a Potent Anti rinderpest Serum and (ii) to set up a Powerful Active Immunity

The procedure customarily adopted at Muktesar until recently was the same as is generally followed still in rinderpest serum stations elsewhere in the world,

three weeks they were bled for the first time for serum then injected under the skin with a massive quantity of highly virulent blood, and bled again for serum after intervals of eight and ten days This practice of massive injection with virulent blood followed by bleeding was repeated for an indefinite period of time although experience had taught us seven years ago that it was not profitable to retain the buffalo producers for bleeding over a longer period than three months, the frequent supervention of abscess formation at the seats of injection and rapid wasting in

bodily condition were deemed to be sufficient reasons for disposing of the animals after this interval

Numerous large controlled experiments executed at Muktesar during the course of the last six years have convinced us that so far as it concerns the Indian buffalo and Himalayan hill bull the term hyper immunization is a misnomer when it is implied that this process of repeated injection of massive quantities of virulent blood tends in any degree to drive up the anti body content of the circulating blood above that which follows the mere application of the initial immunization

When Indian buffaloes that are susceptible to rinderpest are injected with a minute quantity of virulent blood and simultaneously with a controlling amount of anti serum the anti body development in the circulating blood seems to vary in degree and duration according to the following principles —

(i) When the reaction to infection is entirely suppressed by the administration of an excess dose of serum there follows in the animal a solid immunity towards further infection even when it takes the form of a massive injection with virulent blood lasting at any rate for two years The anti body content that is discoverable on test in the circulating blood following upon what may be termed an 'afebrile' 'symptomless, or blocked out' reaction of this kind is minimal This type of reaction has been well studied in laboratory animals by Weil and Breinl with the virus of human typhus In our work the serum obtained *has* some times *displayed negligible protective properties*

(ii) When the reaction to infection is mild but decided' there develops in addition to a powerful immunity a maximal and regular development *of anti bodies in the circulating blood This type of reaction is* manifested by a pronounced thermal response with but slight and often almost inappreciable disturbance in the well being of the animal *The anti body content of the blood rises sharply* on the 18th day after the virus injection to a higher level which is sustained until the 30th to the 36th days Thereafter there is a gradual drop to about half this level or less and the reduced level is maintained with slight but steadily occurring diminution until the 60th to the 90th days

(iii) When the reaction to infection is manifested by decided clinical response, with outwardly visible symptoms of rinderpest the anti body content of the circulating blood develops in the same time relationships as in the above second type of reaction but the curve' indicating its total amount at successive bleedings exhibits marked fluctuations and it does not rise above that produced after the second type at any time

In our experience 'hyper immunization' by repeated injection of massive quantities of virulent blood, has been observed at times with careful tests to sustain partially for many weeks (13 injections) the level of potency of the serum obtained from buffaloes in which the anti body content of the serum

obtained following the original immunization was high and, for some reason whic we cannot explain the serum obtained at certain late bleedings has displayed a unexpectedly high potency With hill bulls treated in this way, the serum h shown invariably a sharp drop in potency after the second hyper immunizatio and further injections have had no effect in restoring it to its original level

We may probably explain the phenomenon of anti body response by assumin that after the initial infection with the minute dose of infective blood, there occu a maximum diffusion of the virus throughout the body, where it propagat apparently in the reticulo endothelial apparatus If the assistance given to t cells of this apparatus in combating the proliferation of the virus is so considerab that it is destroyed before it is liberated into the general circulation to evoke febrile response the subsequent anti body secretion ' from the cells will be co respondingly suppressed With a saturation of the cells with the virus to t extent however that they are not seriously injured there will occur a subseque maximal ' secretion of anti bodies The introduction under the skin from tir to time afterwards of large quantities of blood which may in reality contain aft all minute numbers of the virulent germs in comparison with those which propagat in the predilection seats at the time of the initial diffusion can only be followed by response that is small relative to that which followed the original infection our tests upon the infectivity of what we consider to be highly virulent blood fro hill bulls we have found quantities as small as 1 10 000 c c virulent though wi smaller quantitie it has usually not been found possible to infect If a single ger can prove infective such highly virulent blood would contain the germs only in t proportion of one germ to 60 to 80 red blood cells

It had been found by long experience at Muktesar that the most suitable anima for the propagation of the virus were the very susceptible Himalayan hill bull the most suitable serum producers are buffaloes Repeated massive inoculation buffaloes with hill bull blood brings about the development in the serum of hæmolyt properties towards cattle blood and on one occasion some accidents occurred whe imported cattle were injected with large quantities of this serum in the course the serum simultaneous inoculation death taking place in some cases as early as tl third day with pronounced hæmoglobinuria

It therefore remained to be decided whether the procedure of so called hype immunization was economically and technically worth while with the anim material available in India for serum production It is recognized that in othe countries conditions in both these respects may be different After preliminary larg trials at Muktesar on the production of anti serum by the immune ' method onl the results were considered so satisfactory especially from the economic standpoin that the old procedure of hyper immunization was entirely abandoned, and for th last three years our serum supplies have been manufactured nearly entirely at ou plains branch laboratory near Bareilly, throughout the year, from buffaloes whic are immunized by the injection of a small quantity of virulent blood (despatche from Muktesar) and, on the following day, a carefully estimated (from previou

bodily condition were deemed to be sufficient reasons for disposing of the animals after this interval

Numerous large controlled experiments executed at Muktesar during the course of the last six years have convinced us that so far as it concerns the Indian buffalo and Himalayan hill bull the term hyper immunization is a misnomer when it is implied that this process of repeated injection of massive quantities of virulent blood tends in any degree to drive up the anti body content of the circulating blood above that which follows the mere application of the initial immunization

When Indian buffaloes that are susceptible to rinderpest are injected with a minute quantity of virulent blood and simultaneously with a controlling amount of anti serum the anti body development in the circulating blood seems to vary in degree and duration according to the following principles —

( ) When the reaction to infection is entirely suppressed by the administration of an excess dose of serum there follows in the animal a solid immunity towards further infection even when it takes the form of a massive injection with virulent blood lasting at any rate for two years The anti body content that is discoverable on test in the circulating blood following upon what may be termed an afebrile symptomless or blocked out reaction of this kind is minimal This type of reaction has been well studied in laboratory animals by Weil and Breinl with the virus of human typhus In our work the serum obtained has some times displayed negligible protective properties

(ii) When the reaction to infection is mild but decided there develops in addition to a powerful immunity a maximal and regular development of anti bodies in the circulating blood This type of reaction is manifested by a pronounced thermal response with but slight and often almost inappreciable disturbance in the well being of the animal The anti body content of the blood rises sharply on the 18th day after the virus injection to a higher level which is sustained until the 30th to the 36th days Thereafter there is a gradual drop to about half *this level or less and the reduced level is maintained with slight* but steadily occurring diminution until the 60th to the 90th days

(iii) When the reaction to infection is manifested by decided clinical response with outwardly visible symptoms of rinderpest the anti body content of the circulating blood develops in the same time relationships as in the above second type of reaction but the ' curve indicating its total amount at successive bleedings exhibits marked fluctuations and it does not rise above that produced after the second type at any time

In our experience hyper immunization ' by repeated injection of massive quantities of virulent blood has been observed at times with careful tests to sustain partially for many weeks (13 injections) the *level of potency of the* serum obtained from buffaloes in which the anti body content of the serum

obtained following the original immunization was high and, for some reason which we cannot explain, the serum obtained at certain late bleedings has displayed an unexpectedly high potency With hill bulls treated in this way, the serum has shown invariably a sharp drop in potency after the second hyper immunization, and further injections have had no effect in restoring it to its original level

We may probably explain the phenomenon of anti body response by assuming that after the initial infection, with the minute dose of infective blood, there occurs a maximum diffusion of the virus throughout the body, where it propagates apparently in the reticulo endothelial apparatus If the assistance given to the cells of this apparatus in combating the proliferation of the virus is so considerable that it is destroyed before it is liberated into the general circulation to evoke a febrile response the subsequent anti body 'secretion' from the cells will be correspondingly suppressed With a saturation of the cells with the virus to the extent however, that they are not seriously injured there will occur a subsequent maximal 'secretion of anti bodies The introduction under the skin from time to time afterwards of large quantities of blood which may in reality, contain after all minute numbers of the virulent germs in comparison with those which propagated in the predilection seats at the time of the initial diffusion can only be followed by a response that is small relative to that which followed the original infection In our tests upon the infectivity of what we consider to be highly virulent blood from hill bulls, we have found quantities as small as 1 10 000 c c virulent though with smaller quantities it has usually not been found possible to infect If a single germ can prove infective such highly virulent blood would contain the germs only in the proportion of one germ to 60 to 80 red blood cells

It had been found by long experience at Muktesar that the most suitable animals for the propagation of the virus were the very susceptible Himalayan hill bulls the most suitable serum producers are buffaloes Repeated massive inoculation of buffaloes with hill bull blood brings about the development in the serum of hæmolytic properties towards cattle blood and on one occasion some accidents occurred when imported cattle were injected with large quantities of this serum in the course of the serum simultaneous inoculation death taking place in some cases as early as the third day with pronounced hæmoglobinuria

It therefore remained to be decided whether the procedure of so called hyper immunization was economically and technically worth while with the animal material available in India for serum production It is recognized that in other countries conditions in both these respects may be different After preliminary large trials at Muktesar on the production of anti serum by the 'immune' method only, the results were considered so satisfactory especially from the economic standpoint, that the old procedure of hyper immunization was entirely abandoned and for the last three years our serum supplies have been manufactured nearly entirely at our plains branch laboratory near Bareilly throughout the year, from buffaloes which are immunized by the injection of a small quantity of virulent blood (despatched from Muktesar) and, on the following day a carefully estimated (from previous

xperience with its use) dose of serum so that the desired 'mild but decided eaction is evoked Subsequently bleeding for serum commences on the 18th day ind is continued every three days until the 30th day (L' serum) The procedure hen is to rest the animals six to eight days and bleed three times on alternate days, his is done on three occasions (M N and O) The serum first obtained (L' erum) is generally highly potent and can be used for the serum simultaneous noculation It is also used for mixing with the serum from the later bleedings n the proportions indicated by careful animal tests at Muktesar to furnish the arge brews which are now made up of preventive serum for use in the serum alone nethod of protection

In one of the most recent tests carried out to corroborate the impressions obtained from our earlier tests an adequately large batch of buffaloes was subjected o the new immune method of treatment for serum production concurrently similar batch treated initially in the same way was hyper immunized' according o the older method and bled during the period the immune' animals were ielding serum a third batch was repeatedly injected with small quantities of irulent blood mixed with sterile tapioca grains according to a method recently uggested by a French worker in anti toxin production The sera obtained at the uccessive bleedings from each batch were exhaustively tested and the results ndicated clearly that the sera obtained by the immune method were no less potent han those obtained concurrently with the hyper immune method There was no dvantage whatsoever it appeared in resorting to the expensive process of hyper mmunization

In current practice at our plains substation the buffaloes after yielding nti rinderpest serum are for some time past used afterwards to produce serum gainst hæmorrhagic septicæmia and in any case sold afterwards at a remunerative rice

It may be mentioned parenthetically that we have found also in the course of xtensive experiments that the rationale underlying the process of anti rinderpest erum manufacture seems to apply also very largely to the manufacture economically nd expeditiously of an anti bacterial serum namely the serum which is manufac ured on a large scale at the laboratory for use against the pasteurellosis known as ovine hæmorrhagic septicæmia Injection of an adequate initial dose of bacterial ulture is followed by a maximal development of anti bodies which can be discovered n the blood withdrawn from the body nine days after the injection A culture of rganisms that have become so much attenuated by prolonged cultivation outside he animal body as to produce no clinical symptoms whatever is fully as efficacious s a highly virulent one the effects of which are controlled by the simultaneous dministration of a suitable dose of anti serum The maximal response is provoked y the injection of 100 to 200 c cs of a fairly rich broth culture less than this uantity is insufficient while more does not bring about an additional response With repetition of the injections the buffaloes will yield a serum of sustained potency or a long time (we have tested it for 37 injections) On account of the frequent

development of abscesses at the seat of injection with the later inoculations, it is prudent to discontinue the buffaloes after the fourth injection

It is understood that with the use of what is called anatoxin workers are now able to manufacture an anti toxic serum on much the same lines Our conceptions regarding the theory of anti serum production and hyper immunization would therefore seem to stand in need of some revision

For the production of an anti rinderpest serum therefore the initial reaction needs to be graded so that it is of the mild but decided type The question remains what is the reaction to be aimed at in the current active immunization of *cattle in the field by the serum simultaneous method so as to avoid to the largest* extent possible danger to the lives of the inoculated animals and to confer upon them the highest degree of immunity attainable A very large experience has shown that cattle undergoing a distinct clinical reaction to rinderpest infection are not unlikely to suffer sometimes from serious complications attributable to the resuscitation of dormant internal parasites notably piroplasms (*Babesia bigemina*) and coccidia Cattle in India are almost universally carriers of these parasites, infection is contracted naturally when the animals are young and subsequently they do not appear to suffer any visible ill effects from their persistence in minute numbers within the body An attack of rinderpest seems to bring about a specific depression of the tissues which have held the multiplication of the parasites in check, and hence one may witness an animal succumb in passing through an attack which would probably otherwise not have ended fatally and a careful examination reveals the fact that there has been a large multiplication of piroplasms or coccidia To prevent the specific depression which relaxes the restraint of the tissues upon the multiplication of the dormant parasites the reaction to be achieved ought therefore to be of the symptomless or blocked out kind We have found that a solid immunity follows this type of reaction in young calves and that the duration of the immunity extends at any rate over a period of two years subsequent to the lapse of which period it can be freshened up by the inoculation of virus alone It remains to be determined whether the immunity conferred is of a longer duration in older animals and in animals which pass through a more decided reaction on immunization

Action of Certain Inorganic Salts in Increasing the Anti body Content of the Serum

Researches upon this problem were commenced soon after the appearance of an article by Madsen (1923) reporting the experience gained over some years in the State Serum Institute Copenhagen upon the effects of repeated injection of minute quantities of certain inorganic salts into the circulatory system in stimulating the 'secretion of anti bodies From the charts published by him it would appear that the potency of an anti diphtheria serum obtained from horses, undergoes a gradual increase when the animals are subjected after receiving a hyper immunizing dose of toxin to daily intravenous injection of manganese chloride

In our earlier investigations at Mukteswar tests were conducted upon hill bulls and buffaloes that had been subjected to the procedure of hyper immunization then in current use for the production of anti rinderpest serum The groups of animals were treated with uniform daily doses of gramme molecular solutions of the chlorides of certain kat ions (manganese zinc magnesium barium copper) and also with pilocarpine Although the 'secretory' stimulation was not very marked or consistent in its occurrence in the various experiments the effects produced by zinc and magnesium seemed at least to rival that produced by manganese After several experiments had been conducted upon the action of individual salts the optimum period for applying the non specific treatment and the rate of increase in anti bodies following upon a single intravenous injection the idea was conceived that the 'secretory' phenomenon as between the animal tissues and the so called anti bodies might be allied to the phenomenon of secretion by the tissues in general in so far as it is well known to be affected by the action of inorganic salts In other words *it was not unlikely that if salts other than those indicated were administered* in what may be termed equivalent pharmacological or toxicological dosages the results might be similar A mixture was thus made of nine of the chlorides of the kat ions amongst those of most common occurrence in the body tissues (sodium potassium calcium barium iron magnesium, zinc manganese copper) the proportion of each salt corresponding with the relative toxicity of the salt to the other salts upon intravenous injection as determined by animal experiment The nine salt solution has now been tried out fairly extensively in the routine practice of serum production The estimated dose is injected intravenously two hours before bleeding for serum this dosage produces also very soon an appreciable laxative effect upon the bowels While specific anti body secretion is still active in the body the injection of a single dose at this interval prior to bleeding seems to have an appreciable effect in increasing the anti body content of the serum When the secretion has become relaxed after the lapse of a longer interval subsequent to the rinderpest reaction the salt injection is ineffectual in raising the anti body content In other words the salting of the serum producers when they are still yielding a good serum seems to cause them to furnish a somewhat better serum later, when they are yielding a poor serum the salting does not increase materially the quality of the serum

The Supervention of Piroplasmosis in Cattle undergoing Active Immunization

In animals that are subjected to the serum simultaneous inoculation a distressing complication caused by piroplasmosis (*Babesia bigemina* infection) may be set up in two ways —

(i) By the introduction of the piroplasms with the virulent blood used in the inoculation,

(ii) By the resuscitation of piroplasms that already exist in a latent state of activity in the bodies of carrier animals

The first type of piroplasmosis the inoculated type is seen in animals that have not been infected previously with the piroplasms when the virus is obtained from cattle that are carriers of these parasites Hence the type may be expected to arise in valuable imported cattle and great care has to be exercised to take timely measures to forestall its development It arises after an incubation period of six to twelve days and the onset of the disease is manifested by a sudden sharp rise in temperature The symptoms displayed afterwards are those well known to occur in tropical redwater Prompt intravenous inoculation with trypanblue as soon as a febrile disturbance is detected at any time between the sixth and twelfth days after the injection of the virulent blood is effectual as a rule in preventing the development of serious symptoms There are also certain districts in India where the cattle escape natural infection through the bites of ticks at an early age and in these places one should be careful to keep at hand supplies of trypanblue for injection to control the complication if it should arise in the course of immunization

The second type of piroplasmosis the resuscitated type has already been touched upon earlier in this paper in dealing with the type of rinderpest reaction desirable in animals subjected to the serum simultaneous inoculation It may arise at any time after the development of the rinderpest reaction and frequently brings about rapid collapse in the animal during the earlier part of the reaction or aggravates severely the depression of the animal during what might be otherwise a hopeful convalescence The symptoms are often obscure and usually different from those seen in the inoculated piroplasmosis

We have also observed an extraordinary exaltation in virulence of the small piroplasms (*Theileria mutans*) found almost ubiquitously in the blood of Indian cattle when virulent ox blood is used for the inoculation of imported stock The piroplasm becomes exalted to such a degree that death occurs with symptoms and lesions resembling those of East Coast fever and the presence of Koch's blue bodies in the lymphatic tissue and large mononuclear cells of the blood The disease is similar to that described some years ago by the Russian observers Dschunkowsky and Luhs as having been caused by *Theileria annulata* No specific treatment is known for this complication

With the diversion of the rinderpest virus into the bodies of goats it has become now quite easy to circumvent the difficulty arising through inoculated piroplasmosis The piroplasms are all highly specific parasites and so diversion of the rinderpest virus into goats estranges it from the bovine piroplasms This subject has been dwelt upon briefly in the preceding paper (*Rinderpest Some Properties of the Virus etc*) (*See* pp 711 712)

DISCUSSION

Col A J Williams R A V C (B India) Drew attention to certain apparent breakdowns in the immunity which followed inoculations by the serum simultaneous method and suggested that they might be instances of defective technique in immunization

Mr V Krishnamurti Ayyar (Madras) Two different kinds of sera a serum of high potency for the 'serum simultaneous' method and a serum of low potency for the 'serum alone' method are issued from the Muktesar laboratory I would like to be enlightened as to whether we are justified in using the serum of low potency in the field outbreaks I fully realize that in the case of the 'serum simultaneous' method there is the surety of the animals getting the infection as *the virus is actually introduced into the animals*, while, in an outbreak in the field there is not that surety In an outbreak there may be animals which might not get the infection at all, while there may be others which might be exposed to infection and thus to the same danger as they would be if they had been inoculated by the 'serum simultaneous method Are we justified in using the serum used for the 'serum alone' method in these outbreaks? Will that serum be potent enough to protect the susceptible animals in the outbreak to the same degree as the serum of high potency in animals which are subjected to the 'serum simultaneous' method of inoculation? If it is *contended that in the 'serum-simultaneous method even the* least chance of risk should be avoided, and that the resuscitation of parasites is prevented by using a serum of high potency does not the same reason stand also in the case of field outbreaks even though the number of animals that might naturally get the infection in an outbreak is very small in proportion to the others that might not get infected? Even if it is a single animal are we not justified in protecting it to the same degree as with the serum simultaneous' method? In no protective serum has such a distinction been made although the doses may vary depending upon the degree of susceptibility of the individual cattle to infection I would like to be enlightened on these points

Mr F Ware I V S (Madras) During some simultaneous inoculations in a herd of bullocks in Madras in March 1927 two half bred cases which were standing in the same yard were given 5 c cs of rinderpest virus alone These animals had been protected against this disease previously on some date prior to 1923 and showed absolutely no reaction to the above injection although the reactions amongst the rest of the animals in the yard were severe

My memory may be at fault but I seem to remember that the Egyptian workers found that whereas *young calves when protected by the simultaneous method against* rinderpest did not acquire a long immunity, more mature animals did

Mr J T Edwards (United Provinces) replied Mr Krishnamurti asks me to explain the necessity for issuing two or three different kinds of anti rinderpest serum from Muktesar as is done at the present time The sera designated as Class 1 and Class 2 *for the active immunization of cattle* have been specially prepared for issue during *recent years in increasing quantities*, but still in small proportion to the total output They are sera which are of specially high potency, from the first (L) bleedings of the buffalo produced and which on testing *according to our standard methods block out* or nearly block out a rinderpest reaction in hill bulls when inoculated simultaneously with virulent blood at the rate of 90 c cs per 600 lbs body weight The serum issued for preventive inoculation by the 'serum alone' method is still issued with the same guarantee as in former years, namely, that it protects hill bulls with certainty from death when it is given at the rate of 90 c cs per 600 lbs body weight We have taken special pains however, in recent years by the manufacture of large brews the components of which are put to rigorous test to ensure that all issues of sera for the

preven ive inoculation are of approximately uniform potency so that field workers may gain confidence in the case of the serum in combating outbreaks by merely varying the dose rate when they are confronted with differences in virulence of outbreaks and susceptibility of animals to be protected The serum is certainly somewhat cheaper to produce than the classes of higher potency which in reality are now issued probably at an appreciable loss However, from the point of view of the field worker the administration of a dose of serum the anti body content of which will just protect the animal immediately after inoculation has been shown to be followed by a period of passive immunity which lasts nine days the administration of a much larger dose or of the same dose of a stronger serum is followed by a period of protection which lasts at most only two or three days longer Hence there is not much advantage in using up highly potent sera for the serum alone method of protection

Col Williams refers to the apparent breakdowns in immunity following upon the serum simultaneous inoculation and believes them to be caused by the defective technique applied to the inoculated animals of certain years While this may be so the worker must be on his guard in the light of present knowledge not to commit himself to the expression of unjustifiable optimism concerning the duration of the immunity The Military Dairy records in question so far as I have been able to study them give cause for some apprehension

RESOLUTIONS PASSED AT THE GENERAL BUSINESS MEETING OF THE SEVENTH CONGRESS OF THE FAR EASTERN ASSOCIATION OF TROPICAL MEDICINE

Resolutions put Forward by the Council

1. Proposed by Dr. V. G. Heiser (U.S.A.). Seconded by Col. P. Damrong (Siam).

> 'It is the recommendation of this Council that at future Congresses the hotel and touring expenses of the delegates should be borne either by the delegates themselves or by their Governments and not by the Government of the country issuing the invitation.'

2. Proposed by Dr. A. R. Wellington (I.M.S.). Seconded by Col. J. D. Graham (B. India).

> 'That this Council recommend to the General Meeting that the Congress be held triennially instead of biennially and Article No. 9 (1) of the Constitution be suspended so that it can take effect immediately.'

3. Proposed by Major H. Stott (U.P. India). Seconded by Dr. A. L. Hoops (S.S.).

> 'That a triennial subscription of three pounds sterling be paid in one lump sum.'

4. Proposed by Major A. P. Hitchens (Philippine Islands). Seconded by Major J. J. Harper Nelson (Punjab, India).

> 'It is moved that the Chair appoint a Committee of five with power to decide where the next Congress of the F.E.A.T.M. shall be held.'

The members of the Committee appointed under Resolution 4 were as follows:—

Dr. Wu Lien Teh (North China)
Major A. Parker Hitchens (Philippine Islands)
Dr. J. J. Lonkhuijzen (Netherlands East Indies)
Bt. Col. S. R. Christophers (B. India)
Professor S. Hata (Japan)
Dr. O. Deggeller (General Secretary and Treasurer)

Amendments to the Constitution and By-laws Necessitated by the above Resolutions

Constitution

Article 8 (1) * * * * for 'biennial' read 'triennial' for 'two pounds sterling' read 'three pounds sterling'
Article 9 (1) and (2) For 'biennial' read 'triennial'

By-laws

Chapter 1 Section 4 Chapter 2 Chapter 3, Section 1 and Chapter 4 Section 1 For biennial read 'triennial'

Resolutions Passed by Scientific Sections

(A) Resolutions presented by the joint session of the Expert Plague Committee of the Health Organization of the League of Nations and the Far Eastern Association of Tropical Medicine (*See* Vol II page 130)
(B) Resolutions drawn up by Scientific Section No 4 of the F E A T M Seventh Congress on Malaria (*See* Vol II, page 865)

Election of General Secretary and Treasurer and Local Vice Presidents and Secretaries

The appointment of Dr Deggeller as the General Secretary and Treasurer for the ensuing three years was also accepted unanimously

The Vice Presidents and Local Secretaries elected for the ensuing triennial period were as follows —

Australia	*Vice President*	Dr R W Cilento
	Local Secretary	Dr A H Baldwin
British India—		
Government of India	*Vice President*	Lieut Col F P Mackie
	Local Secretary	Lieut Col J Cunningham
Assam	*Vice President*	Lieut Col J Morison
	Local Secretary	Major T D Murison
Bengal	*Vice President*	Maj Genl G Tate
	Local Secretary	Lieut Col A D Stewart
Bihar & Orissa	*Vice President*	Col W S Wilmore
	Local Secretary	Lieut-Col W C Ross
Bombay	*Vice President*	Lieut Col R. W Anthony
	Local Secretary	Lieut Col. W M Houston
Burma	*Vice-President*	Lieut Col W H C Forster
	Local Secretary	Lieut Col E Bisset

MADRAS	*Vice President*	Lieut Col F W C Bradfield
	Local Secretary	Lieut Col A J H Russell
PUNJAB	*Vice President*	Major J J Harper Nelson
	Local Secretary	Lieut Col C A Gill
UNITED PROVINCES	*Vice President*	Col R F Baird
	Local Secretary	Lieut Col C L Dunn
BRITISH NORTH BORNEO	*Vice President*	Dr P A Dingle
	Local Secretary	Dr H T Conyngham
CEYLON	*Vice President*	Dr J F L Bridger
	Local Secretary	Dr S T Gunasekera
CHINA—		
MANCHURIA	*Vice-President*	Dr Wu Lien Teh (Provisional)
	Local Secretary	Dr Lim Chia Swee (Provisional)
NORTH CHINA	*Vice President*	Dr Shisan C Fang
	Local Secretary	Dr C E Lim
CENTRAL CHINA	*Vice President*	Dr W L New
	Local Secretary	Dr Way Sung New
SOUTH CHINA	*Vice President*	Dr Lee Shu Fan
	Local Secretary	Dr Su Ping Lin
FEDERATED MALAY STATES	*Vice President*	Dr A R Wellington
	Local Secretary	Dr A Neave Kingsbury
FORMOSA	*Vice President*	Dr T Horiuchi
	Local Secretary	Dr S Yokogawa
HAWAII	*Vice President*	Dr C B Cooper
	Local Secretary	Dr F E Trotter
HONGKONG	*Vice President*	Dr J B Addison
	Local Secretary	Dr W B A Moore
INDO CHINA	*Vice President*	Dr M L R Montel
	Local Secretary	Dr F H Guerin
JAPAN	*Vice President*	Dr M Nagayo
	Local Secretary	Dr Y Miyagawa
KOREA	*Vice President*	Dr K Shiga
	Local Secretary	Dr M Ito
KWANTUNG	*Vice President*	Dr I Inaba
	Local Secretary	Dr Y Kuno
MACAO	*Vice President*	Dr P da Costa
	Local Secretary	
NETHERLANDS INDIES	*Vice President*	Dr J J Lonkhuijzen
	Local Secretary	Dr O Deggeller

PHILIPPINE ISLANDS	*Vice President*	Dr Arturo Garcia
	Local Secretary	Dr L Lopez Rizal
PORTUGUESE INDIA NOVA GOA	*Vice President*	Col I Froilano de Mello
	Local Secretary	Dr Roque de Souza
SARAWAK	*Vice President*	Dr E M Marjoribanks
	Local Secretary	Dr W Kusel
SIAM	*Vice President*	H S H Prince Thavara
	Local Secretary	Col Phya Damrong
STRAITS SETTLEMENTS	*Vice President*	Dr A L Hoops
	Local Secretary	Dr J W Scharff
SUMATRA	*Vice President*	Dr H Vervoort
	Local Secretary	Dr W Kouwenaar
UNITED STATES OF AMERICA	*Vice President*	Dr S B Grubbs
	Local Secretary	Lieut Col Silver
ROCKEFELLER FOUNDATION	*Hon Advisor to the Council*	Dr V Heiser

EXHIBITION SECTION.

ARRANGED BY

LIEUT COL A D STEWART, I.M.S.,
Hon Secretary Treasurer for British India

EXHIBITION SECTION.

THE Exhibition was divided into two sections Scientific and Commercial The former was housed in parts of the Medical College the School of Tropical Medicine and the Carmichael Hospital the latter in the Anatomical block of the Medical College

The primary object of the *Scientific* Exhibition was to place before the Congress some of the special and general conditions of medicine, surgery and public health as they exist in India Recognizing the vastness of the subject and the limitations of time and space at the Congress, an attempt was made to illustrate conditions and work more or less peculiar to India, and especially to show as far as possible the scientific work on tropical diseases which is being carried on in India under various organizations The Scientific Exhibition was divided into the following sections —

1 Medical —
   (*a*) Exhibits shown by departments and institutions
   (*b*) Exhibits arranged on special subjects
   (*c*) Exhibits illustrating papers read at Congress
2 Dental
3 Veterinary

The *Commercial* Exhibition aimed at showing the various surgical and medical appliances and preparations *issued and manufactured by firms or branches of firms* resident in India

Both exhibitions were open to members throughout the sessions of the Congress in Calcutta

SCIENTIFIC EXHIBITION.

MEDICAL.

Exhibits shown by Departments and Institutions.

LEAGUE OF NATIONS HEALTH SECTION, EASTERN BUREAU, SINGAPORE.

Four charts relating to —

1 The origin and activity of the Eastern Bureau
2 The movements of Pilgrims and Emigrants in the Far East
3 Distribution of Plague in the East since 1894
4 Epidemiological information and the control of ship borne infection
5 The organization of the League of Nations
6 The Health Organization
7 The Epidemiological Intelligence Service
8 Interchange of Health Officers

PUBLIC HEALTH COMMISSIONER WITH THE GOVERNMENT OF INDIA.

A Charts of —

1 British India Population Vital Statistics, 1925 State Medical Colleges and Schools 1925
2 British India Institutions for Medical Relief in 1925
3 British India State Research Pasteur Hygiene, Lymph, Vaccine and other Medical Institutes 1925
4 British India Number of cases treated at Pasteur Institutes and anti rabic centres
5 British India Vaccine and sera production
6 *British India Smallpox mortality per million of population, 1868 to 1925*
7 British India Number of primary vaccinations 1875 to 1925
8 British India Provincial expenditure on Public Health in Municipalities in 1925 26

B Air Photograph of Hardwar Fair area 1927
C Map of Hardwar Fair area 1927
D Map of India and Burma (Scale 1 inch = 32 miles)

DIRECTOR OF PUBLIC HEALTH, BENGAL.

1 Complete sets of charts showing for every district in Bengal during the past 35 years the following —

Total births, total deaths total cholera deaths, total smallpox deaths, total fever deaths total rainfall total net cropped area, total area under winter rice, total area under autumn rice, total area under jute, total twice cropped area and total outturn of rice

2 Maps showing the monthly course of cholera epidemics throughout the Presidency during a number of past years

3 Complete set of special pictorial posters used for publicity work

4 Complete set of publications used for publicity work

DIRECTOR OF PUBLIC HEALTH, BOMBAY.

1 Map of Sind showing all areas irrigated by existing inundation canals and *incidence of malaria*

2 Plan of Sind showing all areas to be irrigated by the Lloyd barrage and New Sind canals and existing cultivation in areas not commanded by barrage

Photographs of the different stages of the preparation, purification, preservation and potency testing of vaccine lymph prepared at the Vaccine Institute, Belgaum

1 Chart showing effects of piped water supply on bowel disease, etc, in Sholapur city

2 Chart showing deaths from intestinal diseases in Poona cantonment before and after chlorination of water supply

3 Chart showing deaths from intestinal diseases in Poona city before and after chlorination of water supply

DIRECTOR OF PUBLIC HEALTH, BURMA.

1 Maps indicating —

(*a*) Distribution of yaws in Burma

(*b*) The areas in Burma to which the vaccination and prevention of Inoculation Acts have now been extended

(*c*) Distribution of beri beri in Burma

(*d*) Distribution of plague in Burma

(*e*) Distribution of races in Burma

(*f*) Distribution of food crops in Burma

2 A complete set of publications issued from the Hygiene Publicity Bureau

3 Locally made models of the stegomyia mosquito and of Anopheles (*fuliginosus*)

4 Plans of the recently completed Harcourt Butler Institute of Public Health, the Provincial Vaccine Depot Meiktila, *and the Port Health Station*, Rangoon

5 An exhibit showing methods of registration of vital statistics in backward tracts of Burma

DIRECTOR OF PUBLIC HEALTH, MADRAS.

Epidemiological Maps.

1 *Cholera spot map of Tanjore District*—The whole district forming the delta of the Cauvery is an endemic focus for cholera Spread occurs mainly along the water courses Shows several points of similarity to the Gangetic delta

2 *Annual mortality from cholera*, 1866 1926—Shows a rough six yearly periodicity except in the years when big All India festivals take place within the Presidency

3 *Annual mortality from smallpox* 1866-1926—Shows the mortality in the Presidency from smallpox for the past sixty years and how a slight reduction in incidence is noticeable in recent years with the spread of vaccination

4 *Spread of cholera from a large pilgrim centre* 1921—Shows the important part played by these gatherings in the dissemination of cholera, how the disease travels along the routes by which the pilgrims return

5 *Incidence of plague by districts*, 1898-1925—Most frequently infected districts border on Mysore State, Bombay Presidency and the Nizam's Dominions showing that Madras gets its infection mainly from these three areas

6 *Incidence of cholera by districts* 1880-1925—There are two endemic foci for cholera in the Madras Presidency—the delta of the Cauvery and the delta of the Thambaraparani in the districts of Tanjore and Tinnevelly respectively

7 *Map showing sites of annual fairs and festivals in the Presidency which attract more than* 3,000 *pilgrims*—To demonstrate the large part played in the epidemiology of cholera by these festivals With so many centres distributed over the whole Presidency and with the gatherings occurring at varying periods of the year it is not surprising that cholera is present more or less continually Unless and until these centres are given protected water supplies and an adequate organization is set up to cater for the welfare of the pilgrims epidemics of cholera are bound to be a menace to the Presidency

8 *Route of spread of cholera into and through the Presidency in* 1922 23—Shows how a district is affected from neighbouring districts after a certain interval, probably indicates the part played by contacts and carriers in the spread

9 *Spread of cholera from a large pilgrim centre—Tirupati*—This pilgrim centre attracts devotees from outside the Presidency and is therefore a source of danger to the whole of India Shows how an outbreak of cholera at this centre spreads in different directions even outside the Presidency

INDIAN RED CROSS SOCIETY.

Exhibits illustrating its numerous activities in connection with medical relief and public health in India including methods of public health propaganda, pamphlets, leaflets lantern lectures and slides on public health subjects, etc

BOTANICAL SURVEY OF INDIA.

Government of Bengal Cinchona Productions and Manufacture.

The manufacture of Quinine and Cinchona products as carried out in India

1 *Flowering Branches*—(*a*) *Cinchona Ledgeriana*—the most important species being the richest in quinine Rather a delicate species (*b*) *Cinchona Succirubra*—the

species richest in the other alkaloids, and most used in pharmacy This is also the hardiest species (c) *Cinchona Ledgeriana* X *Succirubra*—a robust species combining the qualities of (a) and (b)

2 *Seeds* (*Cinchona Ledgeriana*) —The seed is very light, there being some 70,000 to the ounce An ounce of seed is therefore sufficient for planting out an area of about 20 acres

3 Specimens of *Root* and *Stem* Of various species of cinchona showing appearance and thickness of bark

4 *Bark* —Separated from the tree, dried and ready for the factory

5 *Bark —Ground and sifted, ready for treatment*

6 *Bark Paste* —The pulverized bark treated with lime and water and made into a homogeneous paste

7 *Bark Caustic Soda Sol and Oil* —The bark paste mixed with caustic soda sol and oil as it exists in the extraction vats

8 *Oil Extract* —The oil with all the cinchona alkaloids in solution separated from the bark sludge

9 *Oil and Acid* —The alkaloid bearing oil treated with dilute sulphuric acid which takes up the alkaloids and sets the oil free for further use

10 *Acid Extract* —The acid solution separated off from the previous mixture, and containing all the alkaloids

11 *The Acid Extract Neutralized —On neutralization with caustic soda, the acid* sulphates of the alkaloids are converted into normal sulphates and crude quinine sulphate precipitates

12 *Crude Quinine Sulphate* —The crude sulphate of quinine centrifuged off from the previous mixture

13 *Crude Cinchona Febrifuge* —A mixture of the residual alkaloids precipitated from the mother liquor centrifuged off the crude quinine sulphate

14 *Quinine Sulphate Powder* —The commercial product with 15 3 per cent water of crystallization, obtained by boiling crude sulphate in water with decolorizing carbon, then filtering crystallizing, centrifuging and drying

15 *Cinchona Febrifuge Powder* —Obtained from the crude febrifuge (Ex 13) by grinding in a disintegrator and sifting through fine mesh sieves.

16 (a) *Quinine Hydrochloride*, (b) *Quinine Bihydrochloride* —Special products, a stage in the manufacture of which is quinine sulphate

17 *Quinine Sulphate —An alkaloid existing in comparatively small quantities* in cinchona bark It has of late gained much in importance as a febrifuge

18 *Cinchonine Sulphate* —A secondary product and an important constituent of cinchona febrifuge

19 *Tablets of Quinine Sulphate* —Containing 4 grains each

20 *Quinine Treatments* —As distributed by the Governments of Bengal, Assam, Bihar and Orissa with corresponding instruction forms

21. *Cinchona Febrifuge Tablets* —A product much in demand on account of its cheapness.

ZOOLOGICAL SURVEY OF INDIA.

LIEUT.-COL R B SEYMOUR SEWELL, I M S, and DR B L CHAUDURI, D SC

1 *The Natural Enemies of Mosquito Larvæ*

(a) Hemiptera—Representatives of almost all the families of aquatic Hemiptera are serious enemies of mosquito larvæ, the chief exceptions being very big forms like the giant bug, *Belostoma indicum*, whose proboscis is probably too thick to pierce a mosquito larva

(b) Coleoptera—The Dytiscidæ Hydrophilidæ and Gyrinidæ are destructive to mosquito larvæ

(c) Odonata—The members of almost all the important families of this order destroy mosquito larvæ

(d) Diptera—Some of the most formidable natural enemies of mosquito larvæ are their own relatives, such as the predaceous larvæ of *Megarhinus*, etc, which readily kill and eat them

(e) Crustacea—Several members of this group readily eat mosquito larvæ such as the common freshwater shrimp *Palæmon lamarrei* and the crab *Paratelphusa spinigera* In the case of the crab, however, the taste for mosquito food decreases with age

(f) Mollusca—Certain molluscs (like *Pila globosa*) appear to destroy mosquito larvæ by polluting the water in which they live with their excreta or slime The pollution appears to be due to the presence of toxic substances produced as by products of putrefaction

(g) Pisces—Fish play an important role in the control of mosquito larvæ Members of the genera *Haplochilus* and *Panchax* are by far the best destroyers of mosquito larvæ Various species are known from different parts of India and Burma

2 *The Mollusc Hosts of the Fork tailed Cercariæ*

Examples of all the known Mollusc hosts in India are exhibited, together with drawings showing the structure of the various forms of fork tailed Cercaria known to infect them The two species of Mollusc that are most susceptible to infection by Miracidia are *Indoplanorbis exustus* and *Melanoides tuberculatus* The true Schistosomes have up to the present time been found infecting only *Indoplanorbis exustus* and *Limnæa acuminata*

3 *The Poisonous Snakes of India*—Representative specimens of all the families and sub families of snakes are exhibited, as well as examples of nearly all the known poisonous snakes of India

Examples are also shown of 'Mimicry' between poisonous and non poisonous species

CALCUTTA MEDICAL COLLEGE.

Department of Pathology.

PROFESSOR MAJOR G SHANKS, I M S

Numerous pathological specimens of all tropical diseases

Medical History.

LIEUT COL. P. A F. BARNARDO, C I E, C B E, I M S,

Principal, Medical College, Calcutta

Illustrating the history of Western Medicine in India Old prints and books by well known medical authors of different periods

DR S C BANNERJI,

Officiating Professor of Physiology

A collection of electro cardiograms illustrating normal and pathological cardiac conditions

Chemical Examiner's Department.

MAJOR T C BOYD, I M S,

Chemical Examiner with the Government of Bengal

Medico Legal Specimens and Chemical processes

(1) Curve showing the average content of cholesterol *in Indian blood in health* and in leprosy and filaria infections

(2) A series of curves showing the excretion rates in the urine of trivalent and pentavalent antimony compounds

(3) Decolorization rate of solutions of methylene blue when mixed with normal and kala azar serums under certain conditions

(4) A specimen of a plant (*Sorgum halepense Pers*) containing a cyanophoretic glucoside Plants containing this type of glucoside are a cause of cattle poisoning

(5) Spectrographs of various metals taken in the arc and also plates showing absorption bands of blood, etc

(6) Various specimens of different kinds of opium and methods of testing.

(7) Microphotographs of fat crystals obtained from chylous urine

SCHOOL OF TROPICAL MEDICINE.

DIRECTOR LIEUT COL J W D MEGAW, C I E., V H S, I M S

New methods of teaching and demonstration in tropical diseases Tick typhus fever, models, charts and maps illustrating the disease.

Epidemic dropsy and beri beri, epidemiological and other charts illustrating these diseases

Department of Protozoology.

PROFESSOR LIEUT -COL. R KNOWLES, I M S

Infective granuloma, sketches of the supposed parasite, microscope slides

Rhinosporidium infection, sketches of infected cases, microscope slides and drawings

Sarcosporidiosis, microscopic specimens, infected meat, sketches, etc

Micro projection drawing apparatus

The influence of the thyroid gland on a protozoal infection, charts, graphs, tables, etc

The tick transmission of fowl spirochætosis, dark ground preparations and drawings
The puzzles and fallacies of stained blood film examination in the tropics, a series of colour plates and the letterpress to them.

Department of Pathology and Bacteriology.

PROFESSOR LIEUT COL. H W ACTON, I M S

Seven Rare Diseases

1 Types of skin lesions partially correlated with hypothyroidism (*a*) Ichthyosis hystrix, (*b*) Tylosis (*c*) Ainhum or banded scleroderma

2 Sarcoids or acquired angiomata Several types recognized and are illustrated by coloured drawings and microscopical slides These sarcoids were fairly common in 1926 following epidemic dropsy

3 Lathyrism A rare type of paralysis It occurs commonly in Central India during famine, and is due to eating khesari dhal (*Lathyrus sativa*) There are two views namely that it is either due to eating this dhal or due to grains of akti (*Vicia sativa*) which contaminate this pulse

4 Fibromatosis of the skin. Showing the relationship of Von Recklinghausen's disease, molluscum fibrosum and dermatolysis The latter condition has been mistaken for elephantiasis due to filaria

5 Geographical tongue, or multiple benign plaques of the tongue This condition is fairly common in India, at present the causative organism is not known The disease causes no symptoms, and its importance lies in the fact that it is generally mistaken for syphilis

6 Erythrodermia The term erythrodermia means redness of the skin In the tropics two distinct types are seen which have hitherto not been properly described Ulnar erythrodermia consists of a redness on the inner side of the palm associated with parathyroid deficiency Facial erythrodermia about the mouth and eyes, is frequently mistaken for leprosy

7 Rhinoscleroma This disease is rare in India, we are indebted to Dr Norrie and Dr Judah for sending us cases for observation and investigation

Epidemic Dropsy

The relationship of the humidity and rainfall to the disease The disease in the rice, illustrated by the water test, by cultures and sections of the rice grain The poisons extracted from the rice, their pharmacological action, and the relationship of the vitamines to these infections

Department of Hygiene.

PROFESSORS LIEUT COL. A D STEWART, I M S,

and

DR. R B KHAMBATA

1 Activated Sludge showing stages of preparation :—
Chemical purification at various stages—(ammonia formation nitrite formation, nitrate formation) Microscopic appearances at various stages Microscopic appearances of 'infected' sludge Types of protozoa found in good sludge

Micro organisms cultivated from active sludge Cultures and microscopic appearances

2 Analysis of fats and oils with special reference to estimation of stearic acid content—Titer test of the fatty acids Phytosterol and cholesterol separation and acetate test Separation of liquid and solid fatty acids Calculation of iso oleic acid content and its importance Presence of catalysts in hardened oils

3 Sedimentation in turbid waters

Varieties of turbidity found in India Turbidity in Hooghly water in the monsoon source of this turbidity and its nature (Map of Ganges area Bihar and Orissa specimens of basalt, gneiss laterite rock and laterite soil)

Clarification of this turbidity —(1) In nature (2) By calcium ions (3) By hydrogen ions, showing optimum pH (4) Effect of OH ions (5) Effect of aluminium ions

4 The iodine value of fats and oils

A comparison of Wijs and Hanus' methods, showing—(1) the effect of time, (2) the effect of concentration

Some common adulterants of fats and oils in India Preparation of the Wijs' solution by comparison with the standard tint of potassium bichromate solution 1 6 per cent

5 Estimation of electro conductivity of water

6 The 'Permutit' water softener

Department of Pharmacology.

PROFESSOR LIEUT COL R N CHOPRA, I M S

1 Collection of drugs indigenous to India

2 Drugs used in the indigenous systems of medicine with their important preparations —(a) Ayurvedic (b) Tibbi or Unani

3 Indigenous poisons

4 Pharmacological apparatus

5 Determination of hydrogen ion concentration conductivity and viscosity by the electrical method

6 Graphs showing the pharmacological action of some of the important drugs investigated by this department

Department of Medical Entomology.

PROFESSOR DR C STRICKLAND

ASSISTANT PROFESSOR DR. D N ROY,

ENTOMOLOGIST MR M. O T IYENGAR

1 The systematic illustration of the great natural groups of Annelids and Arthropods

2 Special exhibits of interesting cases of parasitism In lantern slides, wall drawings cyclo microscopes, wax models, and specimen boxes

Department of Serology.

IMPERIAL SEROLOGIST LIEUT COL R B LLOYD, I M S

1 Bloodstains and the identification of their source (2) Blood grouping (3) Hæmolysis and complement fixation test

Department of Chemistry.

PROFESSOR DR S GHOSH

1 Some of the Indian indigenous drugs analysed in the Tropical School and their Active Principles (a) Those included in the British Pharmacopœia (b) Those included in indigenous systems

2 Isolation of anti beri beri vitamine from rice

3 Amine isolated from germinated khesari

4 Steps in the preparation of urea stibamine

5 Estimation of minute amounts of quinine in blood

6 Quantitative micro methods in research on drugs

Helminthology.

DR P A MAPLESTONE, D S O,

Research Worker in Ankylostomiasis and Helminthology

1 Demonstration of Lane's direct centrifugal flotation machine for diagnosis of hookworm infection

2 Cultures of fæces in wire baskets which allows extraction by the Baermann method without transference of culture to a special sieve The use of this apparatus was explained and demonstrated two or three times during the week

3 Section of small intestine of dog passing through the head of an *Ankylostoma caninum* in situ The mouth capsule of the worm was seen filled with mucous membrane drawn into it, and there was considerable hæmorrhage in the sub mucosa opposite this point

4 Specimens of *Hymenolepis nana* and *Hymenolepis diminuta* both from human beings, showing the great difference in size of these two parasites

5 Mounted specimen of *Echinococcus granulosus*, the dog tape worm which causes hydatid disease in man

6 Mounted specimens and jars of *Fasciola hepatica* and *Fasciolopsis buski* to illustrate the differences in the external appearance and the internal anatomy of these parasites

DR V T KORKE,

Research Worker in Ankylostomiasis under the Indian Research Fund Association

7 Relief map model of Gaya, with specimens of soil from different parts of the district

Leprosy.

DR E MUIR,

Research Worker in Leprosy

1 Charts microscopic slides, models etc, relating to the pathology and the diagnosis of leprosy

2 Charts, diagrams, models photographs etc, illustrating the treatment of leprosy

3 Maps charts etc illustrating the incidence of leprosy in different places

4 Clinical demonstration of diagnosis and treatment of leprosy

5 Demonstrations of rat leprosy

Leishmaniasis.

DR L E NAPIER,

Research Worker in Kala a ar.

(a) Incidence of kala azar during monsoon months

(b) Distribution of kala azar in Calcutta

(c) Sandfly theory of transmission Two species *Phlebotomus argentipes* and *Phlebotomus minuta*

(d) Breeding of *phelobotomus argentipes*

(e) Flagellates in mid gut of *Phlebotomus argentipes*

(f) Resistance to infection —Survey of villages near Calcutta

(g) Blood and serum tests from kala azar —The aldehyde test The antimony test Spleen puncture Cultures

(h) Dermal leishmaniasis

(i) Drugs used in the treatment of kala azar

Diabetes.

DR J P BOSE,

Research Worker in Diabetes

1 Sugar in blood a new and simple method for estimation

2 The variations in the susceptibility of rabbits of different colours and breeds to insulin

Charts showing how the brown Belgian hare type of rabbit is extremely susceptible to insulin while the albino Himalayan types are much more resistant The adrenalin response of these rabbits is exactly the reverse

3 Leucoderma, Chloasma

The adrenalin and insulin response in these two groups of cases are quite opposed Leucoderma cases show a high adrenalin response and a low insulin response The chloasma cases show opposite results

4 Scleroderma, Icthyosis hysterix

Cases before and after treatment The hypo thyroid condition as indicated by the minus basal metabolic rate before treatment, changes to normal or even

plus after thyroid medication along with the disappearance of all physical signs and symptoms

5 Sprue

Glucose tolerance tests in a few cases of sprue showing marked defect in the sugar utilization power Some of these cases showed slight improvement after insulin medication

CENTRAL RESEARCH INSTITUTE, KASAULI.

DIRECTOR BT COL. S R CHRISTOPHERS, C I E, O B E K H P F R S, I M S

Serum and Vaccine Section.

CAPTAIN K R K IYENGAR I M S

I *The Preparation of Prophylactic Vaccines on the Large Scale* Exemplified by Cholera Vaccine

(1) Sterility control of the seed flasks (2) Sowing the flasks (3) Purity control of seeded flasks by cultures and stained films (4) Planting the rolled culture bottles for which purpose ordinary whisky bottles are used (5) Washing off the growth from the bottles (6) Decanting the washings into serially numbered tubes (7) Purity control for the vaccine from each bottle (8) Preliminary carbolization (9) Examination of the purity controls (10) Decanting the sediment from the numbered tubes to make the stock bulk vaccine (11) Standardizing the vaccine by means of Brown s opacity tubes (12) Sterilizing the vaccine with carbolic acid (13) Sterility test (14) Toxicity test (15) Diluting the vaccine to the strength required for the finished product (16) Bottling the finished vaccine by Maynard s apparatus (17) Sealing primary and secondary (18) Preparation of Office Copies of each capsule of vaccine (19) Sterility test of the bottled vaccine (20) Sealing test (21) Labelling and packing vaccine

Each step in the process illustrated by specimens and photographs

II *Preparation of Media*

Preparation of casein broth and agar preparation sterilization and rolling of the whisky culture bottles incubating room for manufacture of vaccines on a large scale

Preparation of heated blood glucose broth for pneumococcus vaccine

Specimens of dried media with method of preparation and use —Nutrient agar Serum, Lœffler's serum Aronson s Teague s Endo s Vedder's Starch McConkey s and Conradi's media Specimens of bacterial cultures showing the use of the different media

III *Preparation of Anti-venin*

Specimens of cobra and daboia venoms and anti-venin made therefrom—Method of preparation, bottling etc

Central Malarial Organization and Entomological Section.

Malaria

BT COL S R CHRISTOPHERS, C I E, O B E, F R S, K H P I M S

and

MAJOR J A SINTON, V C, O B E, I M S

A malaria map of India illustrating the intensity of malaria in different parts of India

MAJOR J A SINTON I M S

(i) Chart illustrating the permanent cure rate produced by the treatment of over 2 000 cases of benign and malignant tertian malaria with the cinchona alkaloids with stovarsol preparations and with plasmoquine

(ii) Demonstration of the apparatus and method used for the enumeration of malarial parasites and leucocytes in the blood of malarial cases by the fowl cell method with special reference to field work

(iii) A simple method for testing the strength of quinine in medicinal solutions containing this alkaloid

(iv) Micro-photographs and camera lucida drawings of *Plasmodium tenue* Stephens

MAJOR G COVELL I M S

Demonstration of Christophers' method for the accurate measurement of splenic enlargement in malaria, with specimens of the results obtained and the forms etc, used in the field

Entomology

BT COL S R CHRISTOPHERS I M S

Exhibit of four portable models of Anopheline mosquitoes enlarged to scale of 100 times normal Made at Kasauli

(i) *Anopheles stephensi* showing internal anatomy with special reference to the carriage of malaria

(ii) Models of *A culicifacies*, *A listoni* and *A hyrcanus*, illustrating especially the markings scaling and attitude of the insects while at rest

CAPTAIN P J BARRAUD

(i) Model of *Stegomyia albopicta* Skuse × circa 100

(ii) *Micro-photographs of the Indian Stegomyia showing the characteristic markings* used in the identification of the different species

(iii) Camera lucida drawings of the male genitalia of various Indian Culicines

BT COL S R CHRISTOPHERS I M S

and

DR I M PURI

Drawings of mosquito larvæ illustrating the different methods of feeding in Anophelines and Culicines and also the direction of the currents set up by the action of the mouth brushes

MAJOR G COVELL, I M S

(i) A collection showing all the different species of Anopheline mosquitoes recorded from India

(ii) Maps of India illustrating the recorded distribution of all the known Indian Anophelines

(iii) Apparatus used for the collection preservation storage and carriage of larval and adult mosquitoes

MAJOR J A SINTON, I M S

(i) Camera lucida drawings of different species of *Phlebotomus* from India with special reference to the morphological characters of diagnostic importance

(ii) A 'safety' entomological pill box suited for the demonstration and study of specimens for class and museum work

Literature

(a) Memoirs issued from the Central Malaria Bureau

(b) Central Malaria Bureau Bulletins

(c) Reprints of papers on malaria entomology, etc, by workers at the Central Malaria Bureau

HAFFKINE INSTITUTE, BOMBAY.

DIRECTOR LIEUT COL. F P MACKIL, O B E V H S, I M S

Plague.

The exhibits were arranged in separate groups so as to give a connected account of the progressive development of our knowledge of plague

Historical—Photographic representations (presented by the Wellcome s Museum) of St Roch and St Sebastian the patron saints of the plague stricken, of the different types of dress worn by rat catchers, plague attendants and plague doctors during the 17th century, illustrations of the plague carts and plague pits used for the removal and disposal of the dead during the great plague of London The exhibits illustrating what the conception of plague and its prevention were before the discovery of the plague bacillus

Geographical distribution of plague—Photographs (copied from the reports of the League of Nations) to show the prevalence of plague in the eastern and western hemispheres and in India during the year 1925 and to emphasize the fact that India is now the most widely affected country Charts showing (a) the annual mortality from plague in the whole of India and in the city of Bombay (b) the annual case mortality from plague smallpox and cholera to point out that during the last thirty years plague caused the largest number of deaths in India

Plague is primarily a disease of rats—Mounted specimens and photographs of rats found in India, *Rattus rattus* (the house rat) *Rattus norvegicus* (the drain rat), *Gymnomus tarius* (the lesser bandicoot), *Bandicoota indica* (the larger bandicoot), *Mus musculus* (the mouse) and *Crocidura cærulea* (the musk shrew) together with synoptic charts to

show the distinctive characters of those animals Specimens of dissected normal and plague infected rats to bring out the pathological changes in organs and lymph glands caused by the plague bacillus and to help in the detection of plague infection by the naked eye appearances

A chart to show the close correlation between epizootics in rats and epidemics in man in Bombay for a period of thirty years

Transmission of plague —The original cages employed by the Indian Plague Commission to establish the fact that plague is transmitted from rat to rat, and from rat to other animals through the agency of the rat flea, photographs to show the various stages in the blocking of the proventriculum of rat fleas after they have fed on infected rats photographs of sections of fleas to show the proliferation of plague bacilli in the stomach and proventriculum of the flea

Bionomics of the rat flea —Photographs to illustrate the different kinds of rat fleas (male and female) their development from the egg to larva, pupa, and imago Also actual specimens under microscopes to show the differential characters of the species with explanatory charts

Maps to illustrate the distribution of different types of rat fleas in the United Provinces and their relation to plague mortality in that part of India, two spot maps showing the numerical distribution of fleas

The Plague Bacillus —Photographs of Kitasato and Yersin, the discoverers of the plague bacillus of plague bacilli in the blood buboes spleen liver and lungs specimens of sections under microscope showing the bacilli in those organs, the cultural characters of plague bacilli on agar broth, and sugar media A painting to show the characteristic formation of stalactites in broth by the plague bacillus

Plague in Man —A series of photographs of plague patients showing the appearance of buboes in groin, axilla and the neck, along with drawings to demonstrate how man contracts plague from rats due to his close association with this rodent in dwellings

Prevention of plague —Charts regarding the various methods now in use for the prevention of plague photographs to show the various factors which contribute to the propagation of plague such as insanitary dwellings, mud houses, accumulation of rubbish, houses with grain stored in them grain shops, etc, which provide food and shelter for the rats For the destruction of rats specimens of the various *kinds of traps now in* use and their respective trapping capacity, of various rat poisons with their relative efficiency *of various kinds of baits and their relative efficacy* Charts to demonstrate how the improved elongated wonder trap with barium carbonate in bajri flour as a bait accomplishes the desired end A chart to illustrate how the destruction of rats has an influence in reducing plague mortality in the city of Bombay

Prophylactic inoculation against plague —Photographs models, and specimens so arranged as to illustrate the various stages in the manufacture of Haffkine's plague prophylactic in Bombay these include a ... of the anti plague vaccine the preparation and ... rimary cultures from a cutaneously infected rat, ... broth, their incubation for six weeks, growth at the end of incubation, purity tests on agar and in sugar media the method of sterilizing the flasks by heat, carbolizing the

vaccine, the method of filling vacuumized sterile ampoules with vaccine, tests for the sterility of the vaccine, aerobic and anaerobic methods of cultivation, and boxes of vaccine ready for despatch

Tables of statistics of inoculation with Haffkine's vaccine in India and effect of inoculation on the case incidence and case mortality as contrasted with the uninoculated population, the incidence of the disease among the inoculated is considerably reduced and that the case mortality among the inoculated who subsequently contract plague is reduced by more than one half

Inoculation outfit—A box containing the various apparatus, such as Kapadia's lamp for sterilizing syringes, antiseptic lotions etc, with instructions as to how to inoculate

Treatment of Plague—The anti-plague sera which are available for the treatment of plague with the addresses of the firms where they could be obtained

KALA-AZAR COMMISSION.

DIRECTOR MAJOR H E SHORTT, I M S

Inquiry under Indian Research Fund Association

The various methods of *breeding sandflies* in the laboratory, with the apparatus used for feeding these upon man and laboratory animals The method used by the Kala azar Commission for *Phlebotomus argentipes* and the method suggested by Col McCombie Young for *Phlebotomus papatasii* Sandflies in various stages of development

Photographs illustrating the *life history of the parasite* of kala azar *L donovani*, parasites in endothelial cells in the peripheral blood, the stages of infection of the sandfly up to the stage where the buccal cavity is blocked by a plug of flagellates the sandfly in the act of feeding when infection of the wound seems inevitable, a fully developed Leishman Donovan body in an endothelial cell 3½ hours after ingestion as a flagellate

Photographs illustrating the *methods of diagnosis and treatment* The technique of spleen puncture and intravenous injection, scenes at various treatment centres in Assam

Photographs illustrating the technique adopted by the Commission in the *attempt to transmit the disease* from infected persons to animals and human volunteers by means of the sandfly, *P argentipes*

Photographs illustrating *typical Assam scenery*

Photomicrographs illustrating the *complete life-history of Monocystis mackiei*, the gregarine parasite of the sandfly

Diagrams demonstrating the *life cycle* of *Leishmania donovani* and the anatomy of the sandfly

Charts showing *the incidence of kala azar* in the various districts in Assam

A large model of a sandfly and a model of an Assamese homestead

PASTEUR TREATMENT IN INDIA.

ARRANGED BY LIEUT COL J MORISON, I M S

1 Photographs showing Pasteur Institutes at Kasauli, Coonoor, Rangoon and Shillong, types of laboratory rooms, and typical groups of patients

2 Care of animals Rabbit cages and stands Types of cage suitable for the tropics

3 Negri bodies

4 The preparation of anti rabic vaccine by Semple s method Inoculation of the rabbits, removal of the brain and spinal cord, preparation of the emulsion of 2 per cent brain and cord in normal saline containing 1 per cent carbolic acid the dilution of the emulsion with an equal part of saline, tests for sterility, administration of the vaccine at an institute

5 Export of the vaccine

Filling capsules (*a*) by Maynard s apparatus, (*b*) by vacuum jar method (Fntrican's)

6 Statistical methods and types of cards employed

Graphs showing comparative mortality, (*a*) with dried cord method, (*b*) with Hogyes' method, (*c*) with Semple's method, (*d*) numbers treated throughout India at Pasteur Institutes and at out centres About half of all persons bitten are treated at out centres

7 Graph showing the change in virulence of the Kasauli fixed virus when first isolated in 1905 and in the year 1926

8 Popular posters on anti rabic treatment

Exhibits arranged on special subjects.

THE BACTERIOPHAGE.

DR F D'HERELLE

(Inquiry under the Indian Research Fund Association)

LIEUT COL. J MORISON, I M S,

Director, Pasteur Institute, Rangoon

THE BACTERIOPHAGE IN SPRUE, DYSENTERY AND CHOLERA

FILARIASIS.

LIEUT COL. SIR FRANK P CONNOR Kt, D S O, I M S

1 Specimens, models and paintings of clinical types of Filarial affections

LIEUT COL. H W ACTON, I M S

2 'Filaria' Geographical distribution, seasonal infection, and the relationship of the temperature and humidity Morbid histology showing how the fatty obstruction is caused The importance of a septic focus of the body giving rise to filarial fever The different stages of lymphatic obstruction differential diagnosis, and the methods used for treatment

BT COL. S R CHRISTOPHERS I M S

3 Models of mosquitoes

MAJOR H H KING, I M S,

Director, King Institute of Preventive Medicine Madras

4 The results of a house-to house survey of human filariasis in Saidapet The tables showed the relative constancy of the proportion of infected *Culex fatigans* (the intermediary host) in houses compared with great variations in the incidence of filarial disease Thirty four per cent of mosquitoes caught in houses were found infected. Micro-photographs showing the different stages of development of *mf bancrofti* in mosquitoes and a high infection of proboscis

Filarial infection of the lizard (*Calotes versicolor*) Dissections showing the natural habitat of the worm (subsequently named '*Conispiculum guindiensis*) micro photographs illustrating the morphology of the worm, the micro-filariæ and developmental forms in *C fatigans* A table giving briefly the results of the experimental transmission of the infection to healthy lizards brought about through this intermediary host

NUTRITION AND DEFICIENCY DISEASES.

LIEUT COL R McCARRISON, C I E, I M S,

Officer in charge of Nutritional Inquiry, Coonoor, South India

(Inquiry under the Indian Research Fund Association)

Diagrams and charts illustrating —

(1) The distribution of goitre in India the different types of endemic goitre, the experimental production of goitre cretinism, parathyroid disease, the eradication of goitre and its histo pathology

(2) The effects of faulty food on the various organs and tissues of the body

(3) The experimental production of beri beri and the relation of rice to beri beri and of vitamin insufficiency to it

(4) The nutritional value of the national diets of India and the relative values of cereals and cereal products

(5) The relation of manganese to growth

(6) The effects of good and bad diets

(7) The experimental production of stone in the bladder and its sequelæ

(8) *The effects of manures and irrigation on the vitamin value of cereals*

RELAPSING FEVER.

LIEUT COL J CUNNINGHAM, I M S,

Director, Pasteur Institute, Kasauli

(Investigation under the Indian Research Fund Association)

1 Photographs illustrating epidemics of relapsing fever in southern India the villages, the type of villager effected, convalescent patients

2 Temperature charts illustrating the various types of relapsing fever in the human subject

3 Experimental relapsing fever in animals temperature charts of the various types of infection in (a) monkeys, (b) squirrels (*Sciurus palmarum*)

4 Photographs and *specimens of the appearances of Sp carteri in stained films*, in the tissues and under the dark ground illumination

5 Specimens and photographs of the head louse and the body louse (*P capitis* and *P corporis*) together with smears and sections showing infections in lice of *Sp carteri*

6 *Serology of relapsing fever*

(a) Specimens and photographs showing 'spontaneous clumping' of spirochætes at the end of attack in experimental infections in animals

(b) Charts illustrating the fact that *a strain of spirochæte serologically distinct* from that causing the primary infection is responsible for the relapses in this disease and showing agglutination curves to the seven different antigenic types of spirochæte so far isolated The charts illustrated the type of curve obtained after (i) *primary attack without a relapse* Progressive anti body formation to the type of spirochæte responsible for the infection only (ii) *Primary attack followed by one relapse* Primary formation of agglutinins to the type of spirochæte responsible for the infection followed

by a second formation of anti bodies specific to the type of spirochæte present in the relapse (iii) *Primary attack followed by two relapses.* Agglutinin curves for the first attack and first relapse similar to those outlined above in (ii) followed by the formation of yet a third anti-body specific to the spirochæte causing the second relapse and resulting in anti bodies specific to the three strains circulating in the blood at the same time (iv) Abnormal forms of attack relapse without evidence of primary attack

(c) Demonstration of the fact that any of the given different serological types of spirochæte isolated can be responsible for the primary attack, one or other of the other types being then responsible for the relapses

The results of actual serological tests illustrated by means of instantaneous photographs of dark ground preparations

(d) Diagnosis of the serological type of spirochæte responsible for the disease in the human subject by means of agglutination tests

CHOLERA.

DR J W TOMB, O B E

and

CAPTAIN G C MAITRA, I M S

(Inquiry under the Indian Research Fund Association)

1 The open bowl method of cultivation of vibrios

2 A quantitative method for estimating indol produced by vibrios in peptone cultures

3 A simple method of isolating cholera vibrios from plate culture

4 Photographs illustrating the insanitary habits and customs of the inhabitants of areas where cholera is endemic

5 The naked-eye characteristics of cholera stools

6 Microscopic specimens showing cellular elements in cholera stools.

7 Stained specimens of vibrios isolated from different sources

DYSENTERIC LESIONS.

DR R ROW O B E M D

SPECIMENS OF ILEOCÆCAL TUBERCULOSIS.

LIEUT COL E W C BRADFIELD, O B E I M S,

Professor of Surgery Medical College, Madras

SURGICAL AND PATHOLOGICAL EXHIBITS.

The Edinburgh University Alumni Association.

I Renal arterial supply and distribution prepared by the colloid corrosion method

II Sections of whole organs for macroscopic and microscopic examination. Prepared in the Laboratory of the Royal College of Physicians of Edinburgh

(1) Four preparations of Carcinoma of the rectum
(2) The uterus and placenta *in situ* from a case of Eclampsia
(3) Whole kidney showing tubercular disease with multiple abscesses and extension of T B foci into the ureter
(4) Child's lung showing pleurisy and pneumonia
(5) Child's lung showing lobar pneumonia of both lobes and well marked infarcts of the lower
(6) Longitudinal section of intussusception of small intestine
(7) Section of both lobes of the thyroid *in situ* on either side of the trachea showing cystic dilation with colloid hyperplasia in one lobe

III Photos of plastic operations on the face

OPHTHALMOLOGY.

LIEUT COL. W V COPPINGER, I M S

and

MAJOR E O G KIRWAN I M S

Specimens 1 Staphylomata 2 Septic ulcer cornea 3 Ulcer and Panophthalmitis 4 Couching Cases 5 Trephined Cases 6 Iridocyclitis 7 Epitheliomata Conjunctiva Cornea Lid 8 Gliomata 9 Saromata 10 Injuries penetrating (wound, etc) 11 Siderosis 12 Glaucoma 13 Calcareous degeneration of lens 14 Hydatid Cysts 15 Trachoma 16 Herpes Ophthalmicus 17 Spring catarrh 18 Macular keratitis 19 Cysticercus cellulosæ 20 Iritis

X-RAY AND RADIOLOGY.

ARRANGED BY LIEUT COL. J A SHORTEN I M S

School of Tropical Medicine, Calcutta.

Cards showing detail of *X* Ray therapy of skin diseases

Medical College, Calcutta.

Series of prints dealing with (*a*) Diseases of bones and joints (*b*) Liver abscess (*c*) Cardiospasm (*d*) General subjects of interest

Government X-Ray Institute, Madras.

Series of prints, dealing with (*a*) Calcified guinea worms (*b*) Calcified artery (*c*) Condition in Madura foot (*d*) Stones in gall bladder kidney, ureter, urinary bladder and urethra (*e*) Calcified kidney (*f*) Calcified pancreas (*g*) Skull condition with reference to pituitary body, blood vessels and intravenous blood pressure (*h*) Various presentations in pregnancy and monsters (*i*) Clinical photos of special tropical conditions (*j*) Foreign body in alimentary tract (*k*) Spine condition (*l*) Perth s disease (*m*) Stricture, œsophagus (*n*) Appendicitis, etc

Exhibits illustrating papers read at the Congress.

Dr. C FRIMODT MOLLER

Eight diagrams showing the influence of excessive climatic heat on tuberculous patients, of immediate and after results of treatment the prognostic importance of fever and bacilli in the sputum, and the relation between the contamination of wells and diarrhœa

Dr BERNARD E READ,

Professor of Pharmacology Union Medical College, Peking

Paper 'Ephedrine A review of more recent Botanical Researches Alkaloidal content of the crude drug and experiments with Ephedrine and Pseudo ephedrine to elucidate their action'

Exhibits of drug specimens and kymograph records

Col. S L BRUG,

Director Geneeskundig Laboratorium Weltevreden

Paper '*Filaria malayi* n sp parasitic in Man in the Malay Archipelago'

Specimens of *Filaria malayi* n sp parasitic in man in the Malay Archipelago

Lieut Col F P MACKIE I M S and Dr N H FAIRLEY

Paper 'Recent work on Sprue'

Specimens and charts

Dr N H FAIRLEY, and Lieut Col F P MACKIE I M S

Paper 'Recent work on Schistosomiasis.

Specimens and charts

Dr ALEXANDER EMANUELOV and Dr MANECK M MEHTA

Paper 'A preliminary note on the bacteriological examination of some discharges from cases of anæmia of pregnancy normal pregnancy and of non pregnant cases of anæmia'

Specimens and charts

Lieut Col K. K CHATTERJEE, M C, I T F

Paper 'Amœbiasis'

Specimens and charts

Dr N NAKOMURA

Paper 'Infectious animal diseases and their control in Japan'

Vaccines and sera used in practice

Dr CHARLES W YOUNG

Demonstration of method of feeding sandflies

DENTISTRY.

Dr CHAS G JOHNS

Some interesting specimens of ancient Indian dental art also modern English and American dental prosthetics of various kinds, obturators for the cleft of the hard and soft palates, inter-dental splint for fractures of the jaws, etc

Dr R AHMED, L D S

Specimens of teeth of the typical pan chewer

VETERINARY EXHIBIT.

BY

Mr J W EDWARDS, I V S,

Director, Imperial Institute of Veterinary Research, Muktesar, U. P

I *Photographs*

(*a*) General view of the laboratory buildings (*b*) A typical out kraal in which animals are housed after purchase, prior to their admission into the inner experimental sheds There are eight of these surrounding the Institute (*c*) A class room at the Imperial Institute of Veterinary Research, Muktesar (*d*) A corner of the pathological laboratory (*e*) The interior of a typical cattle shed (*f*) The autoclave room (*g*) The serum storage room at the Branch Institute, Izatnagar (*h*) The chart room at the Branch Institute Izatnagar (*i*) A typical hill bull suffering from rinderpest (*j*) A typical hill bull as used for serum and vaccine production (*k*) Purchasing buffaloes at a cattle fair near Bareilly (United Provinces) (*l*) Characteristic buffaloes used for serum production (*m*) Healthy buffaloes waiting for immunization (*n*) Examining hill bull virus producers for the mouth lesions of rinderpest (*o*) Serum makers waiting to be bled (*p*) A good type of buffalo (weighing 1 260 lbs) It produced over 6 000 doses of anti rinderpest serum and 5 000 doses of hæmorrhagic septicæmia serum in one year (*q*) Withdrawing virulent blood from a bull (*r*) Bleeding an animal for anti rinderpest serum (*s*) Decanting serum after clotting of blood (*t*) Serum preparation (*i*) Oxalated blood just withdrawn, (*ii*) Oxalated blood after settling for four hours, (*iii*) Plasma, (*iv*) Plasma clotted (*v*) Expression of serum from clot (*vi*) Serum with preservative (*u*) Serum bottling An apparatus by means of which six bottles can be filled at a time in less than a minute (*v*) Preparing a goat for withdrawal of virulent rinderpest blood (*w*) Bleeding a goat for rinderpest virus (at Muktesar) (*x*) Taking rinderpest virus from a goat (at Branch Institute Izatnagar) (*y*) Filling rinderpest virus in ampoules (*z*) Preparation of hæmorrhagic septicæmia serum Immunization of buffalo with culture Bottles containing doses of the avirulent culture used in immunization (*a*) Despatching serum from the

Branch Institute at Izatnagar to Muktesar where it is tested and standardized (@) Transporting boxes of serum (by ponies and mules) from Muktesar to the nearest railhead (Kathgodam situated at a distance of 24 miles) from where they are despatched by rail to customers

II *Pathological Specimens*

(*a*) Macroscopic *Tuberculosis* miliary lesions in the lung, and advanced caseating lesions of lung and glands of animals

Rinderpest ulceration of the lips tongue and pharynx of bulls

Foot and mouth disease ulcers of the tongue and lips of bulls

Glanders nodules in the lung of a donkey

Bovine nasal granuloma lesions in the nasal cavity of a bull

Coccidiosis lesions in the liver of a rabbit

Neoplasms, melanotic sarcoma of the liver of a horse, and papilloma of the clitoris of a mare

Parasitic diseases, habronema tumour from the stomach of a horse, *Syngamus laryngeus* in a bull and *Balbiania gigante* in the œsophagus of a buffalo

(*b*) Microscopic *Neoplasms*, epithelioma and infective sarcoma of the dog

Rinderpest, secondary pneumonia in the goat

Tuberculosis, miliary lesions of the lung advanced lesion in the lung of an elephant and of a bull, lesions of the spleen pleura and mammary gland *Johne's disease*, of the intestine and lymphatic glands

Glanders nodules in the lung *Bursattee*, skin lesion of an equine

Actinomycosis of the tongue of an ox *Bovine nasal granuloma* lesion

Blackquarter lesion of the muscle of an ox *Coccidiosis* of the liver of a rabbit *Parasitic lesions* 'fluke' (*Fasciola hepatica* and *Dicrocelium lanceolatum*) disease of the liver, verminous hepatitis, *Trichina spiralis* in muscle, scolices from an echinococcus cyst tumour due to filaria

(*c*) *General pathological lesions*, equine infectious broncho pneumonia, pericarditis, epicarditis, myocarditis and endocarditis fatty degeneration of the kidney

(*d*) *Blood smears* of *Trypanosoma evansi* *Piroplasma* (*Babesia*) *bigemina* *Theileria mutans* Koch's 'blue bodies' and *Piroplasma canis*

III *Bacteriological Specimens*

I Family Mycobacteriaceæ

(*a*) Genus Mycobacterium 1 *M tuberculosis* (*hominis*) 2 *M tuberculosis* (*bovis*) 3 *M avium* Causative organism of avian tuberculosis 4 *M paratuberculosis* Cause of Johne's Disease of Cattle 5 *M phlei* Occurring in hay 6 *M smegmatis* In genitalia, especially in smegma

(*b*) Genus Corynebacterium 7 One culture of a diphtheroid organism

(*c*) Genus Pfeifferella 8 *P mallei* The glanders bacillus

II Family Bacillaceæ

(*a*) Genus Bacillus 9 *B subtilis* 10 *B anthracis* Cause of anthrax

(*b*) Genus Clostridium 11 *C chauvei* Cause of blackquarter or blackleg in cattle 12 *C oedematous maligni* (*Vibrion septique*) Cause of malignant oedema 13 *C botulinum* 14 *C tetani*

III Family Bacteriaceae

A Tribe Bacterieae

(*a*) Genus Proteus 15 *P vulgaris*

(*b*) Genus Salmonella 16 *S abortus equi* Cause of equine abortion 17 *S pullorum* Cause of 'White diarrhoea' in young chicks 18 *S gallinarum* Cause of fowl typhoid Infectious for all poultry 19 *S enteritidis* (*Gaertner*) Originally found in intestines being associated with meat poisoning Widespread among animals

(*c*) Genus Alcaligines 20 *A abortus* (=*B abortus*, Bang) Cause of contagious abortion in cattle 21 *A melitensis* Cause of Malta fever Recent researches point to a very close serological relationship between the above two species 22 *A broncho-septicus* Incriminated as the causative agent of distemper in dogs

B Tribe Pasteurelleae

Genus Pasteurella 23 *P suiseptica* Cause of swine plague 24 *P boviseptica* Cause of haemorrhagic septicaemia in cattle and other animals

IV Family Actinomycetaceae

Genus Erysipelothrix 25 *E rhusiopathiae* (*B erysipelatus suis*) Cause of swine erysipelas

V Family Streptococceae

Genus Streptococcus 26 Streptococcus associated with strangles in horses

IV *Mycological Specimens*

Blastomycetes 1 *Blastomyces farciminosus* (Cryptococcus of Rivolta) Cause of epizootic lymphangitis in solipeds Hyphomycetes 2 *Trychophyton* sp Cause of herpes (circinate ringworm) in domestic animals, notably in cattle and horses 3 *Aspergillus niger* Cause of mycotic pneumonia, notably in birds 4 *Sporotrichum* sp Cause of sporotrichosis—a form of disease characterized by the formation of nodules abscesses and ulcers of the skin particularly in horses and mules

Culture of organism of pleuro pneumonia

Products manufactured by the Institute

Mallein—For diagnosis of glanders in equines and largely used in Army Remount Depots Several strains of the glanders bacillus enter into the preparation of this product, cultures of these are incubated for over a week killed at a temperature of 100°C, filtered and preserved with carbolic

Human tuberculin—For detection of tuberculosis in cattle Glycerine potato broth culture is incubated for about six weeks at 37°C, sterilized at 120°C then evaporated to 1 10th of its original volume and filtered through Berkefeld and Chamberland candles

Avian tuberculin—For diagnosis of Johne's disease or avian tuberculosis in cattle Cultures are kept in the incubator for a period varying from three to six weeks, and the subsequent procedure is as in the case of human tuberculin (q v)

Anti blackquarter serum—Used for conferring immediate immunity upon cattle in the scene of an outbreak of blackquarter The serum is prepared from buffaloes subjected to a series of injections with blackquarter aggressin extending over a period of three weeks or so The animals are then bled from the jugular vein

Anti-anthrax serum—For protecting cattle and horses in the scene of an outbreak of the disease The serum is extracted from horses and buffaloes treated with one injection of anti anthrax serum and three injections of a culture of low virulence

Blackquarter aggressin—Used as protective agent particularly in localities which are subjected to frequent visitations of the blackquarter disease It confers a more durable immunity than the serum Diseased muscle juice is treated with formalin, sterilized preserved and later precipitated by addition of acids The clear fluid, after its pH reaction has been adjusted is filtered and issued if satisfactory on test

Anti rinderpest serum—Issued to all parts of India and also to foreign countries for control of rinderpest the most formidable scourge of cattle Healthy buffaloes are utilized as serum makers They are first immunized by serum simultaneous method and from the 18th day from the date of the double inoculation are subjected to a series of bleedings from the jugular The sera are tested on susceptible animals and classified and issued according to demands Used both for passive ('serum alone') and active ('serum virus') immunization

Hæmorrhagic septicæmia—For inoculation into apparently healthy cattle and cutting short the disease during an outbreak Buffaloes discontinued from rinderpest serum makers, are first immunized by a massive dose of anti hæmorrhagic septicæmia serum and a fairly large dose of virulent culture and later bled for serum This process is repeated The period of immunity conferred by the serum is short

COMMERCIAL EXHIBITION

ADAIR DUTT & CO, LTD, *Hospital and Laboratory Furnishers, Calcutt* Microscopes, Projection Apparatus, Ophthalmological and Illuminating Outfits ing the new Shadow-free Operation Theatre Lamp Pantophos, Koch and Sterz Equipments, Lautenschlager Incubators, Krupp's Stainless Steel Surgical and Instruments Jena Glassware and various other Surgical Hospital and Lab Requisites

ALLEN & HANBURYS LTD, LONDON, *Manufacturers of Surgical Instrument Hospital Furniture* The latest Surgical Instruments, many of them made in Sta Steel, samples of the London Hospital Ligatures, Hicks' Thermometers and Br Essence of Chicken

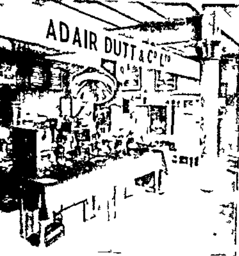
L H ALLISTON & CO, LTD, CALCUTTA AND DELHI, *Agents for Hospital Laboratory Supplies* Scientific Instruments manufactured by The Bausch and Lo Optical Company Rochester N Y Surgical Dressings manufactured by Robinson Sons Ltd, Chesterfield Bohemian Chemical Glassware manufactured by Vladi Josef Kavalier Sazava 'Tycos' Sphygmomanometers manufactured by Short an Mason Ltd, London

THE ANGLO FRENCH DRUG CO (ESTN), LTD, BOMBAY (1) A range of Intravenous products for Intravenous therapy (2) A range of Colloidal products for Injections and Oral uses (3) A range of Organotherapeutic products for both Oral uses and for Injections

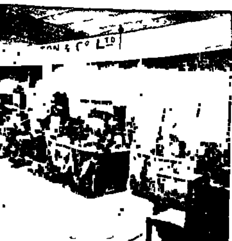
BEHRINGWERKE A G MARBURG-LAHN (GERMANY) *Agent Mr J Mayr Behring Institute* —Institute's preparations, Yatren 105 Yatren Casein, Gono Yatren, Neuro-Yatren, etc Illustrations and coloured diagrams to give clear views of the action of the preparations in the human body A picture showed the Conqueror of Diphtheria and Tetanus Emil von Behring Founder of the Institute Sera of all kinds in 'Serules' and 'Venules' for drawing blood.

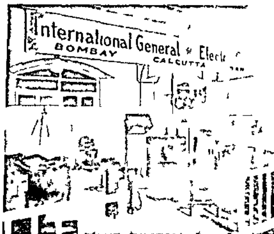
BENGAL CHEMICAL AND PHARMACEUTICAL WORKS, LTD, CALCUTTA, *Manufacturing Chemists* Pharmaceutical tinctures and other products (chemically and physiologically standardized) Acids and other chemicals Surgical dressings Disinfectants Scientific Instruments

THE BENGAL IMMUNITY CO, LTD, CALCUTTA, *Manufacturers of Sera, Vaccines and Organotherapeutic Drugs* Diphtheria anti toxic, Anti dysenteric, Anti strepto-coccal and normal horse sera Polyvalent vaccines Injectules of chemical ... therapeutic preparations (simple and compound) an...
and its oxydases combined variously...

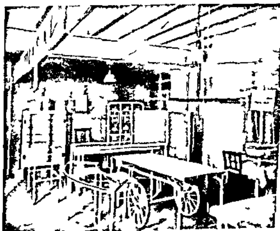

ADAIR DUTT & C° L^TD

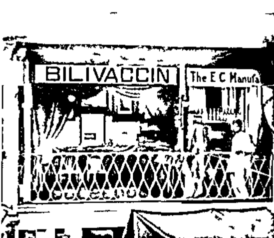




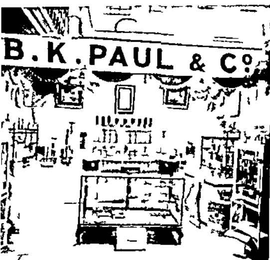

International General
BOMBAY
CALCUTTA


STANISTREET & C° L^TD

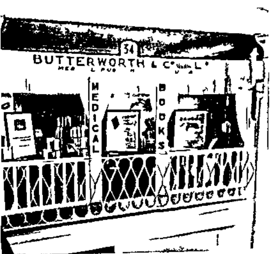


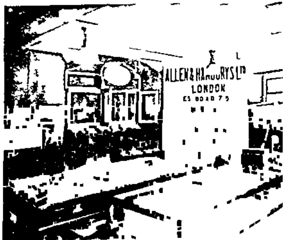

BILIVACCIN
The E C Manufa


THACKER, SPINK


B.K.PAUL & Co


The Scientific Instrument Co Ltd


34
BUTTERWORTH & Co
MEDICAL
BOOKS


LONDON

BOOTS PURE DRUG CO LTD, NOTTINGHAM ENGLAND *Manufacturing Chemists* *Mr R Paxton* A selection of packed specialities such as Regelax Liquid Paraffin Regesan Fruit Saline, the latest products for the treatment of venereal disease such as Acriflavine Sulphostab Bismostab and Thiostab

THE BRITISH DRUG HOUSES LTD LONDON *Mr Henry S Clark Calcutta* Caprokol Radiostol Radio-Malt the new Vitamin Food containing no Cod Liver Oil Ephedrine Hydrochlor Contramine etc also Vaccines and Sera manufactured by the Danish State Serum Works Copenhagen

BURROUGHS WELLCOME & CO LONDON BOMBAY An interesting series of photo micrographs depicting the causative organisms of various diseases Associated with these illustrations were the different medicaments employed in modern practice in the treatment of the diseases which the organisms cause A preparation of recent introduction was shown in Ephedrine Hydrochloride It is issued as a Tabloid product for oral administration and as a Hypoloid product for hypodermic administration

BUTTERWORTH & CO (INDIA) LTD CALCUTTA *Law and Medical Publishers* A wide range of books on Medicine and Surgery such as Sajous Analytic Cyclopædia of Practical Medicine, 6 Volumes and Desk Index The Loose Leaf Practice of Medicine 10 Volumes and Desk Index and Forchheimer Therapeusis of Internal Diseases 6 Volumes, and Desk Index

G W CARNRICK CO NEW YORK U S A BOMBAY *Gland Products* Glandular Products such as Hormotone Secretogen, Trypsogen Kinazyme Vinligen and special formulæ products, as manufactured by G W Carnrick Co

J B DASTOOR CALCUTTA Dr Deschiens products (i) Syrup of Hæmoglobine, (ii) Vin de Hæmoglobine (iii) Tabloid Hæmoglobine manufactured in Paris (France)

MR S N DE CALCUTTA, *Exporter Importer and Manufacturer Manufactured Drugs* —Chaulmoogra Oil Hydnocarpus Oil Pongamia Oil Neem Oil, Garjan Balsam for all kinds of skin diseases and Leprosy

Botanic Drugs —Ephedra Kamala Powder Cinchona Bark Henna Leaves Podophyllum Root and various other African American European and Indian botanicals

DOWN BROTHERS LTD LONDON *Surgical Instrument Makers* 'Down Bros' latest Up to-date model Operation Table Down Bros patent Mono Valve' High Pressure Steam Dressings Sterilizer Electric Instrument Sterilizers Stainless Steel Hospital Furniture Comprehensive selection of instruments including many varieties in stainless steel both for General and Special surgery Instruments for Ophthalmic surgery Electric diagnostic outfits

THE F C MANUFACTURING CO LTD CALCUTTA Electrolytic chlorine of definite guaranteed strength

A K FAZAL & CO CALCUTTA *Chemists and Druggists* The 'Roche' *products* manufactured by Messrs F Hoffmann La Roche & Co Ltd, Basle Switzerland — Quinine Roche Sirolin 'Roche' for diseases of the respiratory system Allonal Roche non narcotic remedy for insomnia and pain Digalen 'Roche' heart *stimulant* Isacen Roche a new laxative Sedobrol 'Roche,' Bromide preparation Thiocol Roche' remedy for affections of the air passages useful in the treatment of tuberculosis, diarrhœas etc. Somnifaine Roche,' Hypnotic and Sedative

H J FOSTER & Co, LTD, CALCUTTA, *Import and Export Merchants* Ostelin (the Vitamin "D" Concentrate), Glaxo, Glaxovo, Glaxo Malted Food

GORDHANDAS DESAI & Co, BOMBAY, *Manufacturers Agents for Hospital and Laboratory Apparatus* (1) Reichert Microscopes, Microtomes, Polarimeters, Photomicrographic apparatus, etc (2) Charles Hearson's Autoclaves, Incubators, Sterilizers and a complete range of bacteriological and biological apparatus (3) Messrs John Weiss's Ophthalmic and General Surgical Instruments and Hospital Furniture (4) Janus Epidiascope and other Projector Lanterns Slides of interest showing Tropical diseases, Sanitation, Child Welfare, Bacteriology, etc

GOVERNMENT OF INDIA CENTRAL PUBLICATION BRANCH, CALCUTTA. A large number of publications of scientific interest showing the scope of the operations of the several Departments under the Government of India, viz, the Agricultural, Archæological, Education, Medical and Sanitary, etc

HAVERO TRADING CO, LTD, HOLLAND The pharmaceutical, biological and veterinary products of 'Bayer Meister Lucius,' Manufactured in the Leverkusen and Hœchst works of Messrs I G Farben industrie Actiengesellschaft, Germany.

HILTON & Co, CALCUTTA, *Chemists and Druggists* Medical books, Medical journals, Surgical instruments, Ultra violet Ray apparatus and stationery, viz, Fountain pen repairing parts

INCELL AND SILK, LTD, CALCUTTA. Aseptic Hospital Furniture and Appliances made in India Amongst these items were Instrument Cabinet, Operation Table, Ambulance Stretcher, Douche Stand, Bed Screen Invalid Chair, Portable High Pressure Sterilizer and Laboratory Sinks

INTERNATIONAL GENERAL ELECTRIC COMPANY, NEW YORK, U S A, BOMBAY The products of the Victor X ray Corporation the Victor Electrocardiograph, Victor Kearsley Stabilizer, Wantz Junior High Tension Transformer Unit, Vario Frequency Diathermy Outfit, and other forms of X ray and Physiotherapy Apparatus

G LOUCATOS, CALCUTTA. *Bilivaccin* Bilivaccin tablets, prepared according to the method of Prof Besredka of the Pasteur Institute of Paris Self vaccination against cholera, typhoid or dysentery The French Product Antivirus, a Broth vaccine dressing for both the curative and preventive treatment of streptococcal and staphylococcal affections

MARTIN AND HARRIS, LTD, CALCUTTA, *Agents for Messrs Merck Wulfing and Co, Ltd* An assortment of Merck's 'Reagent' chemicals and pure pharmaceutical products, M B K Comprettes and Amphioles and Knoll's Specialities (Bromural, Cardiazol, Diuretin, etc), Genasprin, Cystopurin and detoxicated vaccines, Saxon Serum Works Organic Products, Warner's Agarol, Wulfing's Sanatogen, Kalzana, Formamint, Albulactin and Messrs Emil Busch's Microscopes and Ophthalmic Instruments

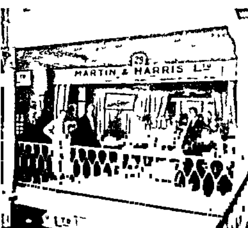
H E METZKES & Co, CALCUTTA, *Manufacturers' Agents for all kinds of Scientific Instruments and Laboratory Requisites including Messrs E Leitz, Wetzlar* Various types of Microscopes with all kinds of accessories, Epidiascopes, Photomicrographic Apparatus Colorimeters, Microtomes, Drawing Apparatus, Microscope Lamps, also various Sterilizers, Autoclaves, Electric and Hand Centrifuges and other bacteriological


SARKAR GUPTA & Co




PARKE, DAVIS & Co


BENGAL TRADES SYNDICATE, CALCUTTA


MARTIN & HARRIS LTD

galenicals, such as tinctures, liquid extracts, etc (4) Albegamn—a new nutrient food (5) Paristan Floating Larvicide for the treatment of tanks, etc, and the destruction of mosquitoes

THACKER, SPINK & CO, LTD, CALCUTTA, *Medical Publishers and Booksellers* Medical books and periodicals including *Indian Journal of Medical Research* and *Indian Medical Gazette* of which they are the publishers A collection of anatomical and pathological models from the Calcutta Model Works

UNION DRUG CO LTD, CALCUTTA *Chemical Manufacturers* A range of organic metalloid compounds, B P tinctures and other indigenous drugs manufactured in their own Laboratories in Calcutta The different stages of organic antimony compounds known as Aminostiburea Stiburea and Novostiburea and the methods of preparation of the organic bismuth compound known as Bisnene for intravenous use

MR. L G VIMAR, CALCUTTA, *Manufacturers and Agents for X ray, Electro medical and Surgical Instruments* X ray and Electro medical apparatus, Surgical instruments and Ultra-violet Mercury lamps

VOLUMES I TO III

INDEX OF AUTHORS

The roman figures w th n bra kets r f r to the pa t ula Volume and the numbe s follo e ng upon them to the pages in the r Tables of Conten s The nam s and fgures pr nted n Ital cs a e refe ences to the remarks made by Authors n the course of the D scuss ons

ACTON H W AND CHOPPA R N (u) 814 (u) 36 444
AHMED P () 459
ANDRE Z (*S e* LABEPNADIL V G F)
APMYTAGE V B GILBEN () *344 3 7 369*
AVARI C R (*See* NAIDU B P B)
AYYAP V KRISHNAMURTI (u) 496 (m) *6°4 651* 658 *661 685 70o 716*

BABLET ET MESNAPD (o48
BAHR P MANSON MAYBUPY L M AND MARTIN P H ( ) 58
PALFOUR M I () 318 *349 8 4*
BALIG1 S () *533*
BANERJI A N L ( ) *567*
BANERJE 4 P K () *301*
BANEPJEE J () 08 ( ) *434*
BANFRJEE I C ( ) *47*
BANIRJI H A) *358*
BANIRJI N ( ) *750*
BANNIRJEI K ( ) 4o7 478 *4o3*
BAI NARDO I A F () I *11 30 5 73*
BAPRALD I J AND COVELL, G (u) 98
BAIILS LI IOY DLS ET HLYMANN () o1
BASU B C (*S e* KNOWLE P AND DAS GUPTA B M)
BASU J B (u) *301*
BASU K I (*55°*
BASU N K (*S e* CHOIRA I N AND GUPTA J C)
BASU S C () *40* ( ) *310*
BASU U I () *7 °(31* ( ) *510*
BATCHELOR M N () *393 406*
BENTLEY C I ( ) *361*
BIIIIITY HILL O () 389 *399*
BHADUPI B N () *5 °9 °8 3 3 316*
BHATIA S L () 143 *I 0*

BISSET E () *483 639*
BOREL, F (m) 165 169
BOSE CHUNI LAL () *308 406 817* ( ) *3 6 347 364*
BOSE J P () *150 193* ( ) *70* (u) 33o
BRADFIELD E W C () ° 1
BPAHMACHARI B B (u) ° 5 *237*
BRAHMACHAPI U N () *6 11* (u) *27* 468 *459*
BROUDIN L (u) 303
BROWNE E HAMILTON () *345*
BRUCE MAYNE (u) 740 745
BRUG S L ( ) *572* (u) °3 9 *310*

CAIUS J F AND NAIDU B P B (u) 481
CARPOLL FAUST ERNEST *S e* EPNEST CAI ROLL FAUST)
CASH J R (*S e* HU C H)
CASH J R AND HU C H ( ) 44 77

CH II A P N ( ) *541* 543 *5 58 586* o*30* (*S e* also ACTON H W)
CHOIRA R N AND GPIWAL, KHEM SINGH (m) 568
CHOPRA R N GUPTA J C AND BASU N K (u) 89
CHOWDHURY UPENII NATH RAY () *01 ° 9*
CHRISTOPHERS S I ( ) 7o6 *780 86 866* ( ) *9 133*
CHRISTOIHERS S R AND IURI I M ( ) 30
CHUNI LAL BOSF (*S e* POSE CHUNI LAL)
CLAUDE, P KELLOGG (*S e* ERNEST CARROLL FAUST)

CLEMESHA, W W (ii) 655, 6?8
CLIVE NEWCOMB (iii) 331 450 42?
CLIVE NEWCOMB AND GANAPATI SANKARAN, (iii) 723
CLIVE NEWCOMB AND YEEDON, P., (i) 270
COMMISSARIAT, S H (i) 830
CONNOP F P., (i) 20? 208 220, 225, 231
COOLTABAWALA, I N., (i) 203, 213
COLLINGE P W N, (i) 2?7, 237, 216
CONVELL, G., (ii) 781 (*See* also BARRAUD P J)
CROW, C G (i) 483
CUNNINGHAM, J., (ii) 2?5 (iii) 536
CUNNINGHAM, J, NICHOLAS, M J AND LAHIRI, B N, (iii) 531

DALAL, P A (ii) 581 (iii) 112
DAS F MAYA, (i) 310 317 (iii) 551
DAS GUPTA, B M, (iii) 34 (*See* also KNOWLES R AND BASU, B C)
DAS, J N, (ii) 301
DAS, KEDARNATH, (i) 310, 3?3, 366
DAVIES, H (i) 395
DE LEON W, (i) 73, 91
DE MELLO I FROILANO, (i) 1?, ?31 535 (ii) 83, ?20 315, 465 483, 582 833
DES BARRES LE ROY (*See* BARRES LE ROY DES)
DEVIDASANI, B J, (i) 3? (iii) 318
DEY, D (iii) 624 6?2, 636
D HERELLE F (ii) 79 219, 231, 278
D HERELLE F, MALONE, R H, AND LAHIRI M N, (ii) 284 288
DHUNJIBHOY, J I, (i) 398 400, 40?
DINH TRI, TRUONG, ET TRINH HUU LOI, (ii) 862
D MONTE D A, (ii) 381
DONALDSON, R S (ii) 361 373
DONATH W F (*See* JANSEN B C P)
DRIVER A H, (i) 3?6
DUGGAN J N, (i) 285 296 306, 307, 310
DUNN, C L (ii) 73 ?19 (iii) 1?7
DUNN C L, AND SAKANDARAM KHAN, (i) 181
DUTT M M (i) 1?3
DUTT, S C (ii) 367

EDWARDS J T, (ii) 507 (iii) 265 327, 452 ?96 598 6?6 64? 651, 65? 65? 664 684 699 705, 707 716
EMANUELOV, A AND MEHTA M M, (i) 379
ERNEST CARL OIL FAUST AND CLAUDE R KELLOGG, (iii) 268

EPLIKAH I S., () 72
ESCH, C D., (i) 29? (ii) 213 5?

FABELLA, JOSE, (i) 817, 8?8
FABIAN HIRST, L., (i) 4?? (…
FAIRLEY, N H (*See* MACKIE, …
FAUST, ERNEST CARL OIL (…) CARL OIL FAUST)
FELICIANO, E T (*See* YEDDE…
FLEMING GOB, P., (i) 3?7
FOLSTER, R H C., (ii) 80 1?…
FLIMODT MOLLER C., (i) 73 …
FROILANO DE MELLO, I (*See* … I FROILANO)
FRY, A B., (ii) 751
FUNAOKA SEIGO (i) 424

GALSTAUN, S G, (i) 429 445 …
GANAPATI PANJA, (i) 136 … 508
GANAPATI SANKARAN (*See* … COMB)
GANGULI, P., (ii) 379 43? (iii) …
GANGULI, S K, (i) 851 (ii) 6…
GANGULY, L B, (i) *11, 32, 40*
GAUTIER R, (i) 781
GHARPURE, P V (iii) *28 625*
GHOSH H, (ii) *421*
GIAN SINGH (i) 687
GILL, C. A, (i) *638* (ii) 624, 826…
GITTINS R J (i) *72* (ii) *43?*, 8…
GLOSTER T H, (iii) 36?
GOHLEN, F H H, (i) 218, 2?8, … 572
GOKHALE S K (*See* SOKHEY, …
GOVIND SINGH THAPAR, (iii) …
GOB, P FLEMING (See *FLE… I*)
GOYLE A N, (ii) *35 12?*
GRAHAM, J D, (i) 463 *491* (ii) …
GREEN ARMYTAGE, V B (… *TAGE, V B GREEN*)
GREWAL KHEM SINGH (*See* … I N)
GUNASAGARAM P K (*See* … RAM, M)
GUPTA, A, (i) 1?? *136* (ii) *37?* (…
GUPTA B M DAS (*See* DAS GUP…
GUPTA J C (*See* CHOPRA, R N, … N K)
GUPTA S K (i) *84?*

HAFFKINE INSTITUTE BOMBAY STAFF OF THE (*See* MACKIE … FAIRLEY, N H)

HAMILTON BROWNE E (See *BROWNE E HAMILTON*)
HANAFIN J B (ii) 7?
HARNETT W I (i) ?00 2?
HARNIE A H (i) *316*
HARPER NELSON J J (i) *11* (ii) *433*
HARUJIRO KOBAYASHI (iii) 18? *2?6*
HARVEY W F AND IYENGAR K R K (i i) 503
HATA SAHACHIRO (i) 458 *488* 5?8
HEADWARDS A (i) 842 *857*
HEISER V G (i) *489* (i) *636* (iii) ?*66* *346* *367*
HENDERSON J M (i) 136 (ii) 34? 355
HERELLE F D (See D HERELLE F)
HERTIG M (See YOUNG C W)
HEWLETT K (iii) *646*
HEYMANN (See BARRES LE ROY DES)
HICKS E P (i) 78 23?
HILL O BERKELEY (See BERKELEY HILL O)
HIRST L FABIAN (See *FABIAN HIRST L*)
HITCHENS A PARKER (i) *41?* *63?* (iii) *536*
HOFFMANN W H (ii) 551
HOOPS A L ( ) *491* (ii) *634* *749*
HOOTON A ( ) 19 ?*03*
HOUSTON B M (i) *490*
HU C H (iii) *23* (See also CASH J R)
HU C H AND CASH J P (i i) 6? 80
HUU LOI TRINH (See DINH TRI TRUONG)

IDA SCUDDER (i) 3?? *35?*
INADA R (i) 437
INTENGAN G (i) 749
ISHIMITSU K (See KATSURADA F AND YOSHINO M)
IYENGAR K R K (i) 5?? (See also HARVEY W F)
IYENGAR M O T (ii) 684 (iii) 11? *1?7* 128 *131* 1?6 *1??*

JAMES S P (i) 60? *638* *78?* *831* *865* *866*
JAMES S P NICOL, W D AND SHUTE P G (ii) 712 ?88
JANSEN B C P (i i) *374* *?67*
JANSEN B C P AND DONATH W F (iii) 37?
JESWIET U P (i) *301*
JOACHIMOVITS R (i) 370
JOLLY G G (i) *4 ?* ( ) *781* (i i) *3?4*
JOSE FABELLA (See FABELLA JOSE)
JOURDRAN E (i) 388 817 821 (ii) 170
JOURDRAN E ET MFILLOUR LE (i) 434
JUNG SHAMSHER (See NAIDU B P B)

KACKER R K (i) *80* (i) *431*
KANJI NAGANO (iii) 211
KAORU MORISHITA (ii) 8?7
KATSUMI MATSUNO (ii) 6 0
KATSURADA F YOSHINO M AND ISHIMITSU K (i) 660
KEDARNATH DAS (See DAS KEDARNATH)
KELLOGG CLAUDE R (See CLAUDE R KELLOGG)
KELSALL R (ii) *3?0* (iii) *367*
KELSER R A STANTON YOUNGBERG AND TEODULO TAPACIO (iii) 6?8
KENDRICK J F (i i) 216 *67*
KERR I (ii) *344* *389*
KESAVA PAI M AND GUNASAGARAM P K (i) 75
KESAVA PAI M AND VENUGOPAL, C A (i) 81
KESSLER A (iii) 409 576, *58*
KHAN SARANJAM (i) *35* (See also DUNN C L)
KHEM SINGH GREWAL (See GREWAL KHEM SINGH)
KING H H (i) 7?? (i i) *309*
KINGSBURY A N [illegible]

554
KNOWLES R DAS GUPTA B M AND BASU B C (ii) ?73
KOBAYASHI HARUJIRO (See HARUJIRO KOBAYASHI)
KORKE V T (iii) 249 ??8 ?67 30? *310*
KRISHNA MENON T (i) *19*
KRISHNAMURTI AYYAR V (See AYYAR V KRISHNAMURTI)
KUBOTA SEIKO (iii) *541* 5?3
KWA TJOAN SIOE (iii) ?00

LABERNADIE V G F (i) 13 17 (ii) 315 (iii) 198 524 5?7 *5??*
LABERNADIE V G F ET ANDRE, Z., (ii) 346
LAHIRI B N (See CUNNINGHAM J AND NICHOLAS M J)

LAHIRI M N (*See* D HEI TLIE F AND MALONE R H)
LAKSHMANASWAMI MUDALIAR 1 () *345 357 850*
I AMBERT A () 788
LANDEMAN F (*See* MUIR F AND WAI D MAN)
LAZARUS H M () *856*
LEGER MARCEL () *415*
LEITCH J N () 641 *8 8 851*
LELE K B () *398*
LEON NORMET () 109
LEON W DE (*See* DE LEON W)
LIEN TEH WU (u) ° *44 129*
LIKUN WEI (u) 791
LITTLE C J H () *°46*
LI YUAN PO () 196
LLOYD R B () *581* () 517 *5°9*
LOI TRINH HUU (*See* HUU LOI TRINH)

MACKIE F P () *° °73*
MACKIE F I FAIRLEY N H AND THE STAFF OF THE HAFFKINE INSTITUTE BOMBAY () °48
MAITRA D N () °40
D I W)
§ 9
AND
LA
L ANG C 74C) 70ɔ
SON)
EL)
e al o
MORISON J)
MARTIN P H (*See* BAHR P MANSON and MAYBURY L M)
MATSUNO KATSUMI (*See* *KATSUMI MAT SUNO*)
MAYA DAS F (*See* DAS F MAYA)
MAYBURY L M (*See* BAHR P MANSON
D H
3 4
3°8 333 343 40 048 0 4ɔ3
455 *457*
McCOMBIE YOUNG T C () *3°* () *185 448*
McGUIRE C (u) 438

McVAIL J B () 477
MEGAW J W D () 509 *516* () °5 349 *368*
MEHTA D H () *19° 851*
MEHTA M M (*See* EMANUELOV A)
MEILLOUP I F (*See* JOURDIAN L)
MELLO I FROILANO DE (*See* DE MELLO I FROILANO)
MENAEL H C () *398* (u) *347*
MENON K KRISHNA (See *KRISHNA MENON K*)
MESNARD (*See* BABLET)
MITRA S (u) *317*
MIYAMOTO TOSINUBO () 667 *68ɔ* 686
MIYAMOTO TOSINUBO NOMURA TOSITUNA AND ONO SIWITI () 665
MODI J J () *45°*
MOLLER C FRIMODT (See *FRIMODT MOLLER C*)
MONTE D A D (*See* D MONTE D A)
MORIN HENRY G S () 5ɔ°
MORISHITA KAORU (*See* KAORU MORISHITA)
MORISON J () *°7° °73 301*
MORISON J AND MARTIN C DE C () °04

341 63
MUIR F () 30. 33° 338 *315* 3 6
MUIR F WAI DMAN AND LANDEMAN E () 36°
MUKERJEE B D () *346*
MUKERJEE J C () *°35*
MUKERJEE S K () 7° °87 *30 30*
MUKHERJI H N () *3°1*
MUKHERJI SANTOSH KUMAR () *41*
MUNSIFF J () 49ɔ *818*
MURPHY M C () *8°9*

NAGANO KANJI (*See* KANJI NAGANO)
NAIDU B P B AND AYARI C R () *96*
NAIDU B P B AND SHAMSHER JUNG (u) 66
NAKAMURA NORICHIKA () *59 6 5*
NAKAMURA YUTAKA () 704
NAPIER L E () *5 488*
NATESAN MUDALIAR C (See *MUDALIAR C NATESAN*)
NEAVE KINGSBURY A (*See* KINGSBURY A NEAVE)
NELSON J J HARPER (See *HARPER NELSON J J*)

NERURKAR K G ( ) *347*
NEWCOMB CLIVF AND SANKAI AN GANAPATI (*See* CLIVL NEWCOMB AND GANAPATI SANKAI AN)
NEWCOMB CLIVE AND VERDON P (*S e* CLIVE NEWCOMB AND VERDON P)
NICHOLAS M J (*See* CUNNINGHAM J AND LAHIRI B N)
NICOL W D (*See* JAMES S P AND SHUTE P G)
NIGAM B P ( ) *81*
NIGAM K S ( ) 190 20 23? 244
NIKANOPOFF S ( ) 84
NISHIO F (*See* ONODERA N YOSHIKI M AND YUKAWA K)
NOMURA TOSITUNA (*See* MIYAMOTO TOSINOBU)
NORICHIKA NAKAMURA (*See* NAKAMURA NORICHIKA)
NORMET LEON (*See* LEON NORMET)
NORONHA A J ( ) *246*
NORRIS R T ( ) *612*
NUNAN W ( ) 408

ONODERA N (n) *591*
ONODERA N NISHIO E YOSHIKI M AND YUKAWA K ( ) 587
ONO SIWITI (*See* MIYAMOTO TOSINUBO)

PAI M KESAVA AND GUNASAGARAM P K (*S e* KESAVA PAI M AND GUNASAGARAM, P K)
PAI M KESAVA AND VENUGOPAL C A (*See* KESAVA PAI M AND VENUGOPAL C A)
PANDIT C G ( ) *235* (m) *309*
PANJA GANAPATI (*See* GANAPATI PANJA)
PAPASURAM G R ( ) 420
PARKER HITCHENS A (See *HITCHENS A PARKER*)
PATEL P T (n) *83* 124 *128*
PENNELL A M ( ) *854*
PHATAK V M ( ) *85?*
PHIPSON E S ( ) 578
PILLAI V D (n) *79*
PINEDA E V ( ) 390 (*See* also WADE H W)
PINTO r

QUIRKE T F (m) *646*

RAHMAN L A ( ) *8 7*
RAMAN TAMII K (n) 3 8
RAMBO I (( ) *36 397*
RAMSAY C (( ) 661
RAO S SUBBA ( ) *01 3? ( ) 55*
RAO S SUNDAR (*18* (n) 09
RAY CH B DHURY UPENDRANATH (e *CHOWDHURY UPENDRANATH RAY*)
RAY I K ( ) *40o*
READ BERNARD E m) 537
ROKURO TAKANO ( ) 580
ROSEDAIL J L ( ) *393 8 6* ( ) *3 6 374*
ROSS H C ( ) *30* ( ) *918 31* ( ) *96*
ROW R (n) 317 *343 86*
ROY CHAUDRI A C (S e *CHAUDRI A C ROY*)
ROY D N ( ) *142*
ROY P C ( ) *347*
RUSHFORTH F V ( ) *313*
RUSSELL A J H ( ) o 3 *31 61 63 518* ( ) 131 *0* (n) *318*
RUTH YOUNG ( ) 8 9 *8 7*

SAHACHIRO HATA (*S e* HATA SAHACHIRO
SAHAI B) *11 30 7*
SANDES J D ( ) 166
SANKARAN GANAPATI (S GANAPATI SANKARAN)
SANTOSH KUMAR MUKHERJI (Se MUKHERJI SANTOSH KUMAR)
SANTRA I ( ) *380*
SARANJAM KHAN (*See* DUNN C I also KHAN SARANJAM)
SARBADHIKARY S ( ) *133*
SARKAR S L ( ) *594 848* ( ) *749* 3 *8 8 866* (n) *24 488 591*
SCHAPPE J W ( ) 613 *748 784* 8?
SCHOBL O (n) *3 9 516* 541
SCOTT A C ( ) 335

( ) *1 6 134 14 135*
SHAHA B ( ) *16* ( ) *31* 8 *831* (n) *363*
SHAMSHER JUNG (*S e* JUNG SHAMSHER)
SHANKS G ( ) *7*
SHARMA I (n) *317*
SHORTEN J A ( ) *433 446*
SHORTT H E ( ) 1 *24* 30 *131*
SHUTE I G (S JAMES S P)
SINGH GIAN (*See* GIAN SINGH)

SINGH GREWAL, KHEM (See GREWAL, KHEM SINGH)
SINGH THAPA, GOVIND (See GOVIND SINGH THAPAR)
SINTON J A ( ) 778 801 *8 0 866* ( ) *1 6 133 17*
SIOE KWA TJOAN (See KWA TJOAN SIOE)
SIWITI ONO (See ONO SIWITI)
SMITH F F STROTHER ( ) 311 *317*
SMITH B O S ( ) *38 38*
SOKHEY S S ( ) 1*01 19*2 ( ) 3 1
SOKHEY S S AND GOKHALE S K ( ) 269
SOKHEY S S AND MALANDKAR M A ( ) 267
SOMERVELL T H (B) *9*
SOPARKAR M B ( ) 4 5 *436* ( ) 603 *6 5*
SPRAWSON C A ( ) 8 *16 192* ( ) *366*
STANTON YOUNGBERG (See KELSER R A AND TEODULO TAPACIO)
STAPLETON G ( ) *348*
STEPHENS J W W ( ) *637 751 786 8*25 ( ) *30*
STEWART A D ( ) 595
STIRLING R F ( ) 647
STOOKES A A ( ) 103
STOTT H (1) *6 11 16 30* 138 *151* 15*2 193* ( ) 266 *366 5*28
STRICKLAND C ( ) *516* 517 *637* 640 7*0*0
STROTHER SMITH F F (See SMITH F F STROTHER)
SUBBA RAO S (See *RAO S SUBBA*)
SUNDAR RAO S (See RAO S SUNDAR)
SURBEK K F ( ) *784* 818
SURJU PRASAD (See *PRASAD SURJU*)
SUR TARAK NATH ( ) 490
SURTI S B ( ) 20 ( ) *8 6*
SWAMINATH C S ( ) *142*
SWEET W C ( ) *785* ( ) 239

TAKANO BOKURO (See BOKURO TAKANO)
TAKASUGI S ( ) 505
TAMPI K RAMAN (See RAMAN TAMPI K)
TANAKA KICHIRO (See *KICHIRO TANAKA*)
TANDAN R B ( ) *31* ( ) 270 *344*
TANIGUCHI TENJI ( ) 490 598 654
TAPACIO TEODULO (See TEODULO TAPACIO)
TARDIEU ( ) 99
TAYLOR *F H* ( ) 143
TAYLOR H A ( ) 447
TAYLOR J ( ) *8 129 273*
TEH WU LIEN (See LIEN TEH WU)
TENJI TANIGUCHI (See TANIGUCHI TENJI)
TEODULO TAPACIO (See KELSER R A AND STANTON YOUNGBERG)
THAPAR GOVIND SINGH (See GOVIND SINGH THAPAR)
THOMSON T O ( ) *516*
THAKUR H S ( ) 844
TIRUMURTI T S ( ) *267 309 31 4 6*
TJOAN SIOE KWA (See KWA TJOAN SIOE)
TOMB *J W* ( ) 37 *39* ( ) *0* ( ) *501 5 3 567*
TOMB J W AND MAITRA C C ( ) 208
TOSINUBO MIYAMOTO (See MIYAMOTO TOSINUBO)
TOSITUNA NOMURA (See NOMURA TOSITUNA)
TRINH HUU LOI (See HUU LOI TRINH)
TRI TRUONG DINH (See DINH TRI TRUONG)
TRUONG DINH TRI (See TRI TRUONG DINH)

UKIL A C ( ) *40* ( ) 22 239 *247 300 394* 409 *48*
UPENDRANATH RAY CHOWDHURY (See *CHOWDHURY UPENDRANATH RAY*)
URCHS O ( ) 51

VACHHARAJANI B C ( ) 297 *310 316*
VEDDER E B ( ) *3 8* 347 *363 646*
VEDDER E B AND FELICIANO R T ( ) 375
VENUGOPAL C A (See KESAVA PAI M)
VERDON E (See CLIVE NEWCOMB)
VYAS B N ( ) *5*

WADE H W AND *PINEDA E V* ( ) 383
WARDMAN (See MUIR E AND LANDMAN E)
WARL F ( ) *6 2 698 716*
WATSON MALCOLM (See MALCOLM WATSON)
WEBB E R ( ) *88* ( ) *431*
WEBB J R D ( ) 653
WEBB M S ( ) 351
WEI LIKUN (See LIKUN WEI)
WELLINGTON A R ( ) *636*
WHITE R SENIOR (See SENIOR WHITE R)

WHITE, S A, (ii) *272.*
WILLIAMS, A J., (iii) *625, 652, 705, 715*
WILLIAMS, D P, (ii) *828, 866*
WILLIAMSON, K B, (ii) 723
WINCKEL, CH W F, (i) 664
WU LIEN TEH (*See* LIEN TEH, WU)

YOSHIKI, M (*See* ONODERA, N, NISHIO, F, AND YUKAWA, K)
YOSHINO, M (*See* KATSURADA F AND ISHIMITSU K)
YOUNGBERG, STANTON (*See* STANTON YOUNGBERG)
YOUNG, C B (ii) *83* (iii) *23*, 30
YOUNG, C W, AND HERTIG, M, (iii) 19 87
YOUNG RUTH (*See* RUTH YOUNG)
YOUNG, T C McCOMBIE (See *McCOMBIE YOUNG, T C*)
YUAN PO, LI (*See* LI YUAN PO)
YUKAWA K. (*See* ONODERA N NISHIO E, AND YOSHIKI, M)
YUTAKA NAKAMURA (*See* NAKAMURA YUTAKA)